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Oxidation of cytochrome c_2 and of cytochrome *c* by reaction centers of *Rhodospirillum rubrum* and *Rhodobacter sphaeroides*. The effect of ionic strength and of lysine modification on oxidation rates

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The oxidation of cytochrome c_2 by the photooxidized reaction center bacteriochlorophyll, P^+-870, in chromatophores of *Rhodospirillum rubrum* can be described using second-order kinetics at all ionic strengths. In a system consisting of isolated *R. rubrum* reaction centers and purified *R. rubrum* cytochrome c_2, the oxidation of cytochrome c_2 also follows second-order kinetics. In both cases, the reaction rates at low ionic strength are weakly dependent on the ionic strength. The data suggest that the cytochrome remains mobile at very low ionic strength, since the observed kinetics can be easily explained assuming no significant tight binding of cytochrome c_2 to the reaction center. In a system consisting of equine cytochrome *c* and reaction centers of either *R. rubrum* or *Rhodobacter sphaeroides*, the cytochrome *c* oxidation rate depends more strongly on the ionic strength. The high reaction rates at low ionic strength suggest that a significant portion of the cytochrome is bound. Using equine cytochrome *c* derivatives modified at specific lysine residues, it was shown that both *R. rubrum* and *Rb. sphaeroides* reaction centers react with equine cytochrome *c* through its exposed heme edge.

Introduction

In whole cells and chromatophores of *Rhodobacter sphaeroides*, the oxidation of cytochrome c_2 by the oxidized photosynthetic reaction center bacteriochlorophyll, P^+-870, exhibits multiphasic kinetics. A fast phase with a half-time of 2 μs is followed by slower phases [1–3]. It has been shown in experiments with purified reaction centers and cytochrome c_2 that the amplitude of the fast phase diminished with increasing ionic strength, while its rate remained unchanged [4]. These experiments and others [3,5] indicate that cytochrome c_2 is tightly bound to the reaction center ($K_d = 1$ μM at approx. 5 mM ionic strength) and does not diffuse freely between different reaction centers. On the other hand, the ionic strength [2] and the viscosity [6] dependence of the slow phase in vivo strongly suggest the involvement of some diffusion process.

Abbreviations: BChl, bacteriochlorophyll; PMS, phenazine methosulfate; P-870, reaction center bacteriochlorophyll

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In *Rhodospirillum rubrum*, oxidation of cytochrome c_2 is slower by approx. two orders of magnitude than in *Rb. sphaeroides* [7]. Experiments in which the rate of cytochrome c_2 photooxidation in *R. rubrum* was monitored as the flash intensity or the viscosity of the medium was varied can most easily be explained by assuming that electron transfer from reaction center to cytochrome c_2 in *R. rubrum* is a diffusional reaction between a mobile cytochrome c_2 and an immobile reaction center [7,8]. In vitro studies on the interaction of *R. rubrum* reaction centers and added *c* type cytochromes have also been carried out [9,10]. For the reaction with either equine cytochrome *c* or *R. rubrum* cytochrome c_2, a diffusion-limited step (complex formation) was followed by the electron transfer. These results were interpreted in terms of an interaction between the cytochrome and the reaction center that is largely electrostatically controlled and involves relatively small portions of the surfaces of the two reactants: a positively charged region on the cytochrome *c* (c_2) and a negatively charged region on the reaction center [9,10].

Considerable structural similarity exists between cytochromes c_2 from photosynthetic bacteria and eukaryotic mitochondrial cytochromes *c* [11,12], as might be expected from the extensive amino acid sequence homology between the two sets of cytochromes. In the case of *R. rubrum* cytochrome c_2, a high resolution X-ray crystal structure reveals extensive similarities in the tertiary structures of this cytochrome and mitochondrial cytochrome *c* [12]. Equine cytochrome *c* can replace cytochrome c_2 as an electron donor to P^+-870 in an in vitro system utilizing *Rb. sphaeroides* reaction centers [4,5,13,14], with kinetic and binding properties very similar to those exhibited by the endogenous *Rb. sphaeroides* cytochrome c_2. Furthermore, evidence suggests that in this system equine cytochrome *c* binds at the same site on the reaction center as does cytochrome c_2 [4]. It has been well documented [15] that all physiological electron-transfer reactions of mitochondrial cytochrome *c* involve electrostatically stabilized complexes between cytochrome *c* and its reaction partners, in which specific positively charged lysine residues on cytochrome *c* interact with negatively charged carboxylate groups on the cytochrome's oxidant or reductant. These lysine residues, which have been highly conserved during the evolution of cytochrome *c*, are all located near the single solvent-exposed heme edge on the so-called front side of the protein. It therefore seemed reasonable to expect that the interactions of cytochrome c_2 with its reaction partners would involve similarly positioned lysine residues on the front side of cytochrome c_2. However, a recent report [16] suggested that reaction centers of *R. rubrum* might bind preferentially to the so-called backside of *R. rubrum* cytochrome c_2 (away from the exposed heme edge). Since the technique used in these measurements cannot distinguish between non-productive vs. kinetically significant complexes [17], it appeared useful to approach the question of which portion of cytochrome *c* is involved in binding to the reaction center using an alternative technique. In this work we have investigated the kinetics of *R. rubrum* cytochrome c_2 and equine cytochrome *c* oxidation by reaction centers of *R. rubrum*, both in chromatophores and with the purified components, at various ionic strengths. We have also studied the interaction of *R. rubrum* and *Rb. sphaeroides* reaction centers with a series of equine cytochrome *c* derivatives covalently modified at specific single lysine residues.

Materials and Methods

Cells of *R. rubrum* (strain S1) were grown anaerobically, in a medium described by Slooten [18] and flushed with a mixture of nitrogen and CO_2 in flat (Roux) bottles in a water bath at 30 °C. The bottles were irradiated with two 150 W incandescent lamps, since high light intensities were shown to give the highest cytochrome per reaction center yield. After inoculation, the cells were allowed to grow for one day. They were harvested by centrifugation and washed once in 50 mM Tricine buffer (pH 8.0) containing 100 mM KCl. After resuspension, the cells were broken in a French press. Debris and large cell fragments were removed by centrifugation. The chromatophores were stored at 5 °C and used within a few days after preparation. Reaction centers of *R. rubrum* were prepared as described in Ref. 19. Reaction centers of *Rb. sphaeroides* R26 were

prepared as in Ref. 20. *R. rubrum* cytochrome c_2 was purified from sonicated cells as described in Ref. 21. Bacteriochlorophyll, P-870 and cytochromes were estimated as described previously [8]. The *m*-trifluoromethylphenylcarbamoyllysine-modified equine cytochromes were prepared as previously described [22]. Absorbance changes following xenon flashes were measured as in Ref. 7. Absorbance changes produced by between 40 and 80 multiple flashes, spaced 10 s apart, were signal-averaged. Ascorbate plus phenazine methosulfate were present during the kinetic measurements to reduce completely, in the interval between flashes, any ferrocytochrome c_2 produced. The levels of ascorbate plus PMS were low enough so as not to affect significantly the kinetics observed immediately after the flash. Second-order rate constants were estimated from computer fits of the kinetic traces.

Results

Cytochrome c_2 oxidation in chromatophores of Rhodospirillum rubrum

Fig. 1A and B shows typical kinetic traces for the photooxidation and subsequent reduction of P^+-870 in *R. rubrum* chromatophores at two different ionic strengths. The amount of cytochrome c_2 present in the chromatophores, which varies somewhat in different chromatophore preparations, was 0.25 per P-870 in these experiments. Thus, after a saturating flash, only 25% of the reaction centers can be reduced by cytochrome c_2 during the first turnover. This corresponds to the fast phase of P^+-870 reduction in both figures. The oxidation of cytochrome c_2 monitored at 550 nm, was equal in rate and extent to that of P^+-870 reduction at both ionic strengths (data not shown). In Fig. 1A the initial rate of P^+-870 reduction is $2 \cdot 10^3$ s^{-1}. An estimate of the value of the second-order rate constant (k_2) for the reaction can be obtained if the internal cytochrome c_2 concentration is calculated by assuming that the cytochrome c_2 is confined to the known internal volume of the chromatophore [6]. For the chromatophore preparation utilized in the experiments of Fig. 1A and B the cytochrome concentration estimated in this fashion was 0.1 mM, yielding a second-order rate constant of $k_2 = 5 \cdot 10^6$ $M^{-1} \cdot s^{-1}$ for the reduction of P^+-870 by ferrocytochrome c_2. In chromatophores, a given reduced cytochrome c_2 molecule can reduce any one of several reaction centers. This is demonstrated by the traces in Fig. 1A measured at lower flash intensity. The fraction of rapidly reduced P^+-870 increased from the 23% observed at saturating flash intensity to 33% at 18% of saturation. This phenomenon, first described in whole cells of *R. rubrum* [7], has also previously been observed in *R. rubrum* chromatophores [23]. At low ionic strength (Fig. 1B) essentially the same result was obtained: the fraction of rapidly reduced P^+-870 increased from 23% to 40% on lowering the flash

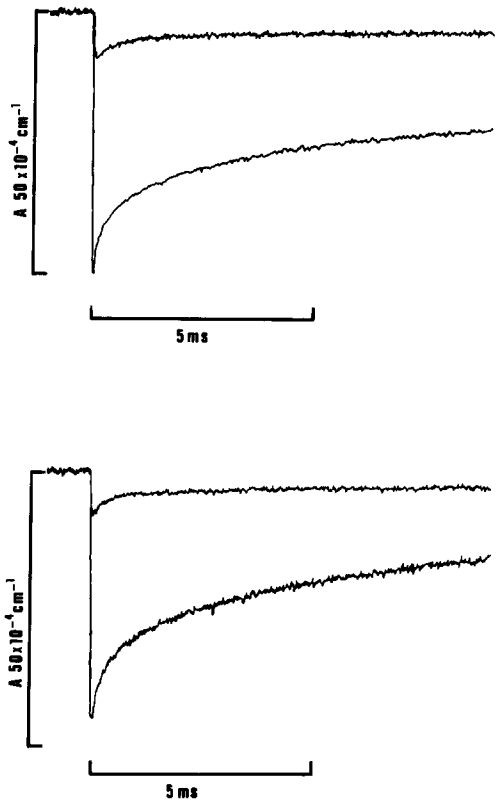

Fig. 1. Time-courses of the reduction of flash oxidized P-870 in chromatophores of *R. rubrum* measured at 604 nm following a weak and a saturating flash. The average cytochrome c_2 content was about five molecules per chromatophore (1 per four reaction centers). The reaction mixture contained chromatophores (equivalent to 1 μM BChl), 1 μM PMS and 2 mM sodium ascorbate in Tris buffer (pH 8.0). Tris concentration was 500 mM and 5 mM for (A) and (B), respectively. The lower traces were obtained at saturating flash intensity while the upper traces were obtained 18% of saturation.

intensity. Similar experiments with *Rb. sphaeroides* chromatophores failed to show this type of cytochrome mobility [5,24].

Fig. 2 shows the rate of cytochrome c_2 oxidation in *R. rubrum* chromatophores as a function of external ionic strength. The ionophore, gramicidin, was added to facilitate equilibration between the internal and external ionic strengths. The data for oxidation of cytochrome c_2 at each ionic strength gave a good fit to the computer-generated curves for a second-order process. The ionic-strength dependence of k_2 was comparable to that observed for the slow phase of cytochrome c_2 oxidation in *Rb. sphaeroides* chromatophores [2] and in reconstituted phospholipid vesicles [25,26]. The decrease in k_2 with increasing ionic strength can be ascribed to a shielding of the opposite charges in the binding regions of cytochrome (+) and the reaction center (−) at higher ionic strength. An unexpected decrease in k_2, observed at very low ionic strengths, will be discussed below.

Cytochrome c_2/c oxidation by isolated reaction centers

As the chromatophore membrane, because of its low permeability to ions, could in principle interfere with adjustment of the internal ionic strength at the site of cytochrome c_2 oxidation by P^+-870, oxidation of cytochrome c_2 and cytochrome *c* by P^+-870 was also studied using reaction centers isolated from *R. rubrum* and *Rb. sphaeroides* R26. The ionic strength dependence of the reaction rate for cytochrome c_2 oxidation by *R. rubrum* reaction centers is shown in Fig. 3. At ionic strengths between 30 and 120 mM, the reaction decreases moderately with increasing salt concentrations. Both the observed rate constant and its dependence on ionic strength are similar to those previously reported [9]. The value at high ionic strength (100 mM or more) is close to that estimated in chromatophores, suggesting that the presence or absence of the membrane does not affect the reaction kinetics to a significant extent. Although the dependence of the second-order rate constant for cytochrome c_2 oxidation on ionic strength is more pronounced than that observed in chromatophores, a fit with Marcus' theory of electron transfer in electrolytes [27] is only possible over a very limited range of ionic strengths (60–150 mM). The product of the charges of the reacting molecules that gives the best fit to Marcus theory is -15 $(esu)^2$, in reasonable agreement with that obtained by Rickle and Cusanovich [9]. Below 60 mM ionic strength, the observation deviates strongly from Marcus' theory. Instead of the pronounced increase in the reaction rate with decreasing ionic strength predicted by the theory, a marked decrease at low ionic strengths occurs (Fig. 3). The effect is similar to that observed in chromatophores (see Fig. 2).

At very low ionic strength *Rb. sphaeroides* cyto-

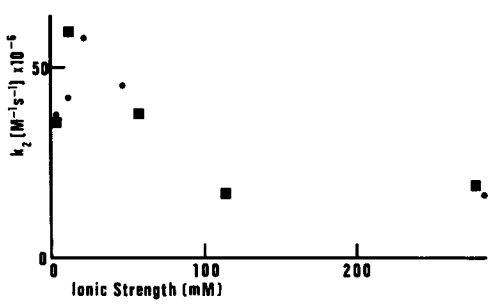

Fig. 2. Effects of ionic strength on the rate of cytochrome c_2 oxidation by P^+-870 in chromatophores of *R. rubrum*. The reaction mixture contained chromatophores (equivalent to 7 μM BChl), 1 μM PMS, 2 mM ascorbate and 4 μM gramicidin in 10 mM Tris buffer (pH 8.0). The ionic strength was varied by changing the concentration of Tris (■) or by adding KCl (●). The individual points were obtained from computer fits to a second-order process.

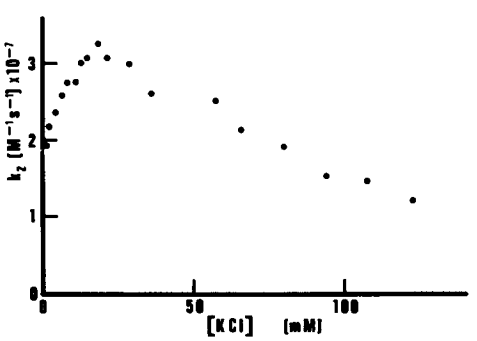

Fig. 3. Effect of ionic strength on the rate of oxidation of cytochrome c_2 by reaction centers of *R. rubrum*. The reaction mixture utilized to obtain these data contained 1 μM *R. rubrum* reaction centers, 5.4 μM *R. rubrum* cytochrome c_2 and 250 μM ascorbate in 10 mM Tris buffer (pH 8.0). KCl was added as indicated.

chrome c_2 and equine cytochrome c are reported to bind strongly to the *Rb. sphaeroides* reaction center [3–5], resulting in a fast phase in the electron transfer from reduced cytochrome to P^+-870. However, we find that with *R. rubrum* reaction centers the oxidation kinetics are second order at all ionic strengths and reactant concentrations tested. Fig. 4 shows typical cytochrome c_2 oxidation and re-reduction kinetics at $I = 2.7$ mM (5 mM Tris buffer) for three cytochrome c_2 concentrations. We have also varied the fraction of P^+-870 oxidized by changing the flash intensity at fixed cytochrome c_2 concentrations. The results of these experiments gave good experimental fits to an equation based on the scheme:

$$P^+ + \text{cytochrome } c_2 \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} + P^+ \cdot \text{cytochrome } c_2 \xrightarrow{k_3} P \cdot \text{cytochrome } c_2^+ \quad (1)$$

proposed by Rickle and Cusanovich for *R. rubrum* [9].

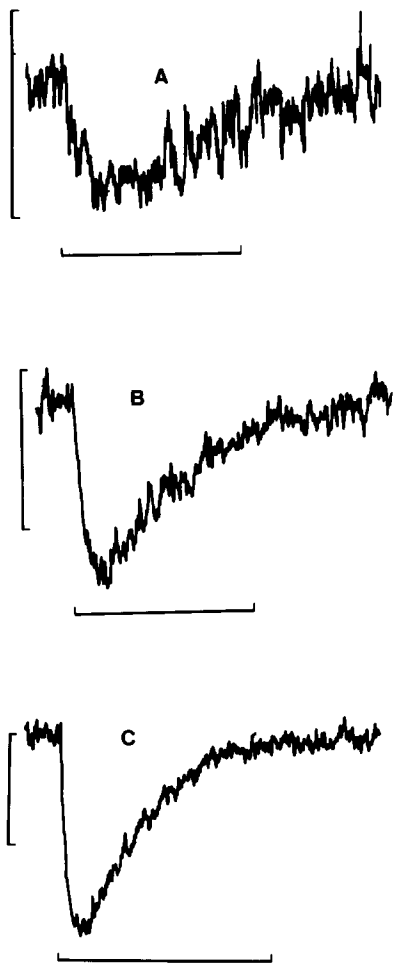

Fig. 4. Kinetic traces of the oxidation of *R. rubrum* cytochrome c_2 by added reaction centers of *R. rubrum* after a flash. The reaction mixture contained 1 μM *R. rubrum* reaction centers in 5 mM Tris buffer (pH 8.0), 200 μM sodium ascorbate, 100 μM 1,4-naphthoquinone and *R. rubrum* cytochrome c_2 at a concentration of 0.14 μM (A), 0.43 μM (B) or 0.86 μM (C). Horizontal bars are equivalent to 100 ms, vertical bars to 50 nM. Cytochrome oxidation was followed at 550 nm. The amount of P-870 oxidized by the flash was 300 nM.

Fig. 5 shows the ionic strength dependence of equine cytochrome c oxidation by *R. rubrum* reaction centers. The reaction kinetics gave good fits to second-order kinetics at all ionic strengths tested. No fast phase due to tightly bound cytochrome c was detected. The ionic strength dependence was more pronounced for cytochrome c oxidation than for that of cytochrome c_2, with a level region at low ionic strength not explainable by Marcus' electron-transfer theory.

Oxidation of equine cytochrome c and specific lysine-modified cytochrome c by reaction centers of Rhodospirillum rubrum and Rhodobacter sphaeroides

To further investigate the factors that govern interactions between cytochrome c and the reaction centers, an attempt was made to identify specific cytochrome c amino acid residues involved in the association of the cytochrome with the reaction center. For this purpose we used

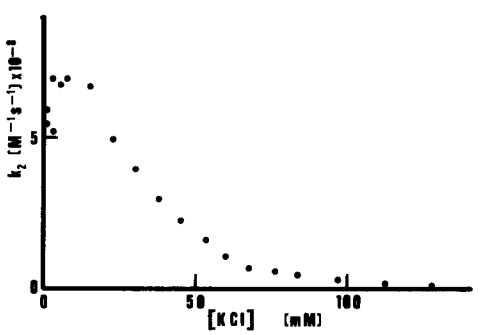

Fig. 5. The effect of ionic strength on the oxidation of equine cytochrome c by reaction centers of *R. rubrum*. The reaction mixture contained 5 μM cytochrome c, 1 μM reaction centers and 250 μM ascorbate in 10 mM Tris buffer (pH 8.0).

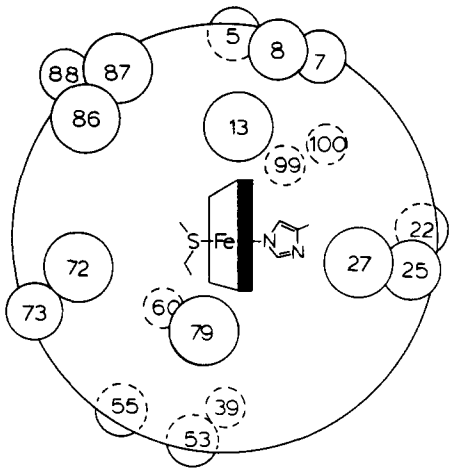

Fig. 6. Location of lysine residues on the surface of equine cytochrome *c*.

equine cytochrome *c* derivatives that had been modified at specific lysine positions (see Fig. 6) to alter their charge from +1 to zero [22]. As can be seen in Fig. 7, Lys-13-modified cytochrome *c* is oxidized by *R. rubrum* reaction centers 7–8-fold more slowly than is the native cytochrome. For *R. rubrum* reaction centers at this ionic strength, the second-order rate constant for oxidation of unmodified equine cytochrome *c* is $5 \cdot 10^8$ $M^{-1} \cdot s^{-1}$, while that for *Rb. sphaeroides* reaction centers is somewhat greater ($1 \cdot 10^9$ $M^{-1} \cdot s^{-1}$). Modification of other front-side lysines, located near the exposed heme edge, also produced significant de-

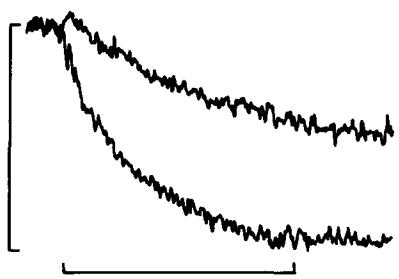

Fig. 7. Oxidation of native and Lys-13-modified equine cytochrome *c* by *R. rubrum* reaction centers. The reaction mixture contained 1 μM reaction center, 1 μM PMS, 500 μM ascorbate and 1 μM cytochrome *c* in 50 mM Tris buffer (pH 8.0). The upper trace is from an experiment using Lys-13-modified cytochrome *c*, while the lower trace is from an experiment with native cytochrome *c*. Measuring wavelength, 550 nm. The horizontal bar represents 5 ms and the vertical bar 500 nM cytochrome. The amount of P-870 oxidized by the flash was 110 nM.

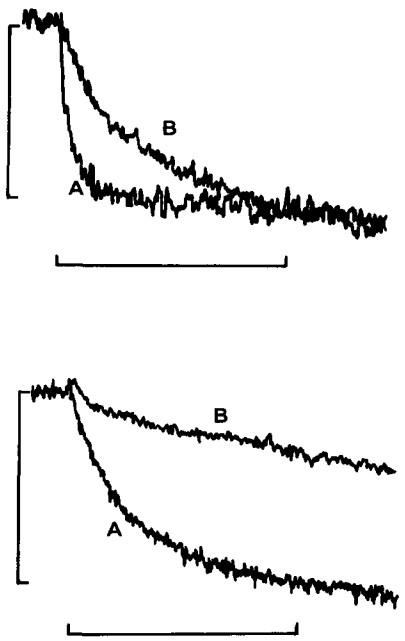

Fig. 8. Oxidation of Lys-13 and Lys-100-modified equine cytochromes *c* by *Rb. sphaeroides* reaction centers. The reaction mixtures contained 1 μM reaction center, 1 μM cytochrome *c*, 1 μM PMS and 500 μM ascorbate in 1 mM Tris buffer (pH 8.0). The upper set of traces represents a set of experiments utilizing Lys-100-modified cytochrome *c* at 1 mM (A) or 30 mM (B) ionic strength. The lower set of traces represents experiments with Lys-13-modified cytochrome *c* at 1 mM (A) or 50 mM (B) ionic strength. The ionic strength was adjusted with KCl. Other conditions as in Fig. 7.

creases in the second-order rate constant for the reduction of P^+-870 by ferrocytochrome *c*. In contrast, modification of a backside cytochrome *c* lysine residue (Lys-100) gave a rate of P^+-870

TABLE I

THE EFFECT OF LYSINE MODIFICATION ON THE OXIDATION RATE OF EQUINE CYTOCHROME *c* BY BACTERIAL REACTION CENTERS

All values were measured in 50 mM Tris buffer (pH 8.0).

Lysine modified	Second-order rate constant ($\times 10^{-9}$ $M^{-1} \cdot s^{-1}$)	
	Rb. sphaeroides	*R. rubrum*
Native	0.85	0.5
No. 100	0.5	0.7
No. 13	0.13	0.07
No. 8	0.2	0.08
No. 72	0.14	0.1
No. 27	–	0.12

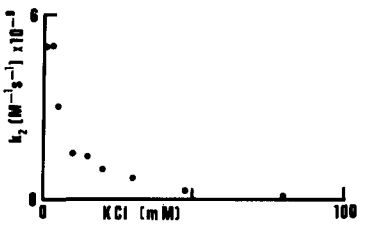

Fig. 9. Effect of ionic strength on the oxidation of Lys-100-modified equine cytochrome *c* by *R. rubrum* reaction centers. The reaction mixture contained 1 μM reaction center, 1 μM cytochrome, 1 μM PMS and 1 mM ascorbate in 1 mM Tris buffer (pH 8.0).

reduction in *R. rubrum* reaction centers that was nearly the same (within the experimental uncertainty) as that observed with native cytochrome *c*. Fig. 8 shows representative kinetic traces for the oxidation of equine cytochrome *c* by *Rb. sphaeroides* reaction centers. The rapid oxidation of lysine 100-modified cytochrome *c* is in contrast to the slower oxidation seen with the Lys-13-modified cytochrome. Table I summarizes the results for a number of cytochrome derivatives in experiments performed using reaction centers isolated from *R. rubrum* and *Rb. sphaeroides* R26. The pattern observed with *Rb. sphaeroides* R26 is similar to that seen with *R. rubrum* except for some inhibition (28%) seen with the lysine 100-modified cytochrome *c*.

The kinetics of cytochrome *c* oxidation (both native and lysine-modified) gave reasonably good fits to second-order kinetics at all ionic strengths tested. However, k_2 was slightly dependent on the cytochrome *c*/*RC* ratio, decreasing approx. 20% at higher ratios. Fig. 9 shows the ionic strength dependence of the second order rate constant for the oxidation of equine cytochrome *c* modified at Lys-100 by *R. rubrum* reaction centers. A similar ionic strength dependence was observed for lysine 13-modified cytochrome *c* (data not shown).

Discussion

Our results show that at all ionic strengths tested the reaction of isolated *R. rubrum* reaction centers with cytochrome c_2 can be accounted for by the reaction scheme (Eqn. 1) proposed by Rickle and Cusanovich [9]. This scheme applies equally well to reaction centers in situ and in the chromatophore membrane. In this model, the relatively low rate of cytochrome c_2 oxidation in *R. rubrum* chromatophores and whole cells is attributed to the high rate constant of dissociation of the reaction center-cytochrome c_2 complex (k_{21}) as compared with the forward electron-transfer reaction rate (k_3). At 30 mM ionic strength, it appears that $k_{21} \gg k_3$. This implies that at the concentrations of reactants used in the experiment described above, the fraction of cytochrome complexed to the reaction center prior to flash excitation was always very low. Thus, the effective reaction rate depends linearly on k_{12}, in good agreement with the observed viscosity dependence of the reaction rate [6]. However, in contrast to the conclusion in Ref. 9, we found that the ionic strength dependence of the cytochrome c_2 oxidation rate both for *R. rubrum* chromatophores and isolated reaction centers is not in agreement with Marcus' theory. The predicted increase in the rate constant at low ionic strength was not observed, indicating that some non-electrostatic effects determine the reaction probability. The importance of this phenomenon is hard to assess, since its extent varied to some degree from preparation to preparation of the reaction centers. A similar phenomenon has been observed in other system such as the reduction of chloroplast P-700 by equine cytochrome *c* [3] and in the oxidation of equine cytochrome *c* and *Rb. sphaeroides* cytochrome c_2 by reaction centers of *Rb. sphaeroides* in negatively charged membranes [23]. In the latter case the decrease in the cytochrome c_2 oxidation rate was ascribed to the retarded lateral diffusion of cytochrome c_2 on the negatively charged membrane.

The oxidation of equine cytochrome *c* by *R. rubrum* reaction centers at low ionic strength is much faster than that of *R. rubrum* cytochrome c_2 and it is possible that a small fraction of the kinetics may correspond to a fast phase, resulting from some cytochrome *c* binding to the reaction center. However, by far the dominant contribution is from a second-order reaction. These properties of *R. rubrum* reaction centers stand in sharp contrast to those of the reaction centers of *Rb. sphaeroides* R26, [3,4]. At low ionic strength and sufficiently high cytochrome concentration both equine cytochrome *c* and *Rb. sphaeroides* cyto-

chrome c_2 show flash-induced oxidation kinetics that are indicative of tight cytochrome binding to the *Rb. sphaeroides* reaction center. Moreover, for reduced cytochrome and reduced reaction centers the dissociation of the cytochrome from the reaction center appears to be very slow. The complicated model accounting for these data [25,26] does not appear applicable to *R. rubrum*.

In the reactions of P^+-870 with equine cytochrome *c* modified at specific lysines, both *R. rubrum* and *Rb. sphaeroides* reaction centers show similar behavior. High rates of reduction of P^+-870 are observed for the unmodified and backside modified (Lys-100) cytochromes, while the front-side modifications all result in markedly lower rates. It is clear that cytochrome *c* with Lys-13 modified is the poorest reductant with both reaction centers. The lysine at position 13 is central in the proposed binding domain of mitochondrial cytochrome *c* to its mitochondrial partners [12,15]. The second largest effect was obtained by modifying Lys-72, which again corresponds well with the proposed relative importance of the lysine at this position for the reaction of cytochrome *c* with its mitochondrial reaction partners [15]. For *R. rubrum* reaction centers, Lys-27 of the cytochrome also appears to be involved in the binding. Somewhat surprising was the large effect of modification of Lys-8 on the reaction of cytochrome *c* with the *R. rubrum* reaction center. The location of Lys-8 on the front side of the cytochrome would be expected to produce some effect of the reaction rate. However, because Lys-8 is somewhat removed from the exposed heme edge (see Fig. 6), the large decrease of the rate observed on modification of this residue suggests the possibility of a more direct role of this residue in the interaction of the cytochrome with the *R. rubrum* reaction center. In contrast, the effect of modification of Lys-8 on the redox reaction with *Rb. sphaeroides* reaction center is only moderate, as is the case with the effect that modification of Lys-8 has on the relative reactivity of the cytochrome with its mitochondrial reaction partners [15].

In summary, lysine residues on the front side of equine cytochrome *c* appear to be involved in the interaction of the cytochrome with bacterial reaction centers, as indicated by the fact that modification of these lysines results in significant decreases in the rate of cytochrome oxidation by P^+-870. Although both *Rb. sphaeroides* and *R. rubrum* show the same general pattern, Lys-8 seems to be of relatively larger importance in binding to *R. rubrum* than to *Rb. sphaeroides* reaction centers. This difference in the binding domain may be one of the causes for some of the different behavior observed in cytochrome oxidation by P^+-870 with the two different bacteria in situ. The results obtained in this study appear to be at variance with those of Rieder et al. [16], which suggested that the backside of *R. rubrum* cytochrome c_2 is involved in binding to the reaction center. One possibility is that the decrease in k_2 observed at very low ionic strength is due to non-productive binding and that the binding domain mapped by Rieder et al. is that involved in non-productive binding. To obtain additional information on this question, it was of interest to extend these investigations from studies with equine cytochrome *c* as a model reductant for P^+-870 to studies with the native cytochrome c_2. Such investigations have recently been completed [10].

Acknowledgements

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