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ASSESSMENT OF THE ROLE OF ELECTROSTATICS IN THE ASSEMBLY OF REACTION CENTER - CYTOCHROME C ELECTRON TRANSFER COMPLEXES

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Abstract Electrostatic forces drive the spontaneous assembly of electron transfer complexes between the photosynthetic reaction center from Rhodobacter sphaeroides and water soluble c-cytochromes. The existence of multiple configurations for the reaction center - cytochrome c complex is reflected in multiphasic electron transfer kinetics. We have measured the electron transfer properties for a series of structurally homologous c-cytochromes. A comparison of electron transfer and electrostatic properties of the cytochromes provides a means of evaluating the role of charged amino acid residues in the assembly of these complexes. These experiments establish that i) the conserved cluster of lysine residues around the exposed heme edge is a necessary but not sufficient condition for formation of an efficient electron transfer complex with the reaction center, and ii) a specific amino acid sequence or "lock and key" mechanism is not responsible for formation of reaction center-cytochrome c electron transfer complexes.

Keywords: Electrostatics, photosynthetic reaction center, cytochrome c, electron transfer complex, assembly, heme proteins

INTRODUCTION

The photosynthetic reaction center from Rhodobacter sphaeroides provides a useful experimental model for investigating mechanisms for assembly of electron transfer complexes with water soluble c-cytochromes. Cytochrome c_2 is the physiological electron donor to the R. sphaeroides reaction center and in vitro kinetics studies have yielded detailed reaction schemes[1-6]. An oxidized cation radical is formed on the reaction center bacteriochlorophyll dimer with a 3 ps rise time following laser excitation[7,8]. This electron hole is filled by electron transfer from cytochrome c_2. The oxidation kinetics are generally seen to be at least biphasic. The component with the fastest reaction time is associated with a reaction center bound cytochrome. The reaction time is about 1 μs, and is independent of cytochrome concentration, while the relative extent does increase with increasing cytochrome concentration following a typical binding equilibrium. The slower kinetic components generally show cytochrome dependent reaction times, indicating some type of diffusional reaction.

The R. sphaeroides reaction center has been shown to react with c-cytochromes from other organisms, including cytochrome c from horse[1-6], yeast[9] and cytochrome c_2

from Rhodospirillum rubrum[9]. Electron transfer with horse cytochrome c is the best characterized. The reaction scheme is found to be equivalent to that for the R. sphaeroides cytochrome, although the electron transfer rate for the fastest component is about 20-fold slower[1-6]. Chemical cross-linking studies suggested that the slowness of the horse cytochrome electron transfer results from binding in a configuration which requires reorganization prior to electron transfer[4]. Optical dichroism measurements provided direct evidence that the two cytochromes are bound to the reaction center in different orientations, and identified the likely binding domains on each cytochrome[10]. These experiments lead to the notion that the structural details of cytochrome c electron transfer complexes will be determined by the different charge distribution for each cytochrome, rather than shared or conserved structural features.

Two models of the reaction center-cytochrome c complex have been built. One used the R. rubrum cytochrome c_2 crystal structure and emphasized complementary charge pairing between residues on the reaction center and lysine residues on the cytochrome surface which are conserved in a large number of c-cytochromes[11]. Another model was built using the charge distribution specific for the R. sphaeroides cytochrome c_2, and maximized the largest number of charge pairs[12].

In this paper we present an experimental test for the role of charged amino acid distributions in the docking of c-cytochromes on the R. sphaeroides reaction center by characterizing the electron transfer efficiencies for a series of structurally homologous c-cytochromes. The role of net charge, dipole moment and surface Coulomb potential in the assembly of these complexes has been evaluated by calculating these electrostatic properties from the available cytochrome crystal structures.

METHODS

Reaction centers from R. sphaeroides were isolated using the detergent lauryldimethylamine-N-oxide (LDAO) as described previously[13]. The cytochromes c from Candida krusei, horse heart, tuna, Saccharomyces cerevisiae, and Pseudomonas aeruginosa were purchased from Sigma, and were used without further purification, except dialysis. The cytochromes c_2 were isolated from R. sphaeroides, R. capsulatus, and Rhodopseudomonas viridis according to the procedures described by Bartsch[14].

The oxidation kinetics of the c-cytochromes were assayed in an argon purged buffer containing 0.025% LDAO, 10 mM Tris, 0.1 mM EDTA, pH 7.8, along with 10 μM phenazine ethosulphate, 20 μM 2-OH-1,4 naphthoquinone, 40 μM ubiquinone-0 and 12 μM ubiquinone-10 as redox mediators. The redox potential of the solution was adjusted to approximately 100 mV with sodium dithionite. The reaction center concentration was

1 μM and the cytochrome concentrations were varied between 1 μM and 100 μM.

Electron transfer kinetics from the c-cytochromes were monitored by the disappearance of the bacteriochlorophyll dimer cation band at 1250 nm following excitation with a 1.5 mJ, 30 ns wide, 590 nm pulse from a Nd:YAG pumped rhodamine dye laser. The excitation and measuring beams were at right angles. The decay kinetics of the 1250 nm band were fit as the sum of 1 to 3 single exponentials, using a Levenberg-Marquardt non-linear least squares fitting program, kindly provided by Dr. Seth Snyder (Chemistry Division, Argonne National Laboratory). Second order rate constants for the concentration dependent components were determined over the range of 1 μM to 10 μM.

The net charge (Σq_i), dipole moment[15] ($\Sigma q_i r_i$) and Coulomb potential[16] ($\Sigma q_t q_i / 80 r_i$) were calculated from the crystal structures of the tuna[17], P. aeruginosa[18] and R. rubrum[19] cytochromes, taken from the Brookhaven Protein Data Bank. The model of the R. sphaeroides cytochrome was built as described previously[12]. The atomic charges, q_i, for lysine, arginine, glutamate and aspartate residues and the carboxy and amino terminals were taken from ref. 20, and charges for the heme atoms were taken from ref. 21.

RESULTS

Table I lists the rate constants for electron transfer between the R. sphaeroides reaction center and a series of c-cytochromes. Assay of the electron transfer kinetics as a function of cytochrome concentration allowed the contributions due to reaction center bound cytochrome components to be distinguished from diffusional components whose oxidation rates were dependent on cytochrome concentration. The cytochromes bound in an electron transfer complexes before the flash oxidation of the reaction center showed first order rate constants which were characteristic of the complex and independent of cytochrome concentration. The table lists the cytochromes from top to bottom in descending order of electron transfer efficacy.

The oxidation kinetics of the R. sphaeroides cytochrome c_2 could be fit as a biexponential decay at all cytochrome concentrations. The electron transfer complex formed with this cytochrome was characterized by a 0.9 μs +/- 0.4 μs reaction time, which reached a maximal extent of about 0.8 with saturating cytochrome concentration. The dissociation constant, K_D, was estimated to be about 1 μM from the cytochrome concentration which the half-maximal extent was seen. The slower kinetic component shows a cytochrome concentration dependent rate which corresponds to a second order rate constant of 1×10^9 M^{-1} s^{-1}.

TABLE I Electron transfer rates between the Rp. sphaeroides reaction center and a series of c-cytochromes.

		cytochrome independent kinetic components					cytochrome dependent kinetic components
	redox	τ_1	A_1	τ_2	A_2	K_D	k_{2nd}
cytochrome	mV	μs		μs		μM	$M^{-1}s^{-1}$
sphaeroides	350	0.9(.4)	0.8	-	-	1(1)	$1(1) \times 10^9$
tuna	260	0.7(.3)	0.4	12(2)	0.4	2(3)	$1(1) \times 10^9$
capsulatus	340	0.7(.1)	0.7	-	-	2(2)	$8(2) \times 10^8$
horse	260	19(4)	0.9	-	-	16(8)	$1(1) \times 10^9$
cerevisiae	260	28(3)	0.9	-	-	1(1)	$1(1) \times 10^9$
krusei	265	47(2)	0.7	-	-		
rubrum	324	-	0.0	-	-		2×10^8 (a)
viridis	300	-	0.0	-	-		$6(1) \times 10^7$
aeruginosa	270	-	0.0	-	-		$< 10^3$

E_h (P/P^+) = 450 mV

(a) J.E. Long, B. Durham, M.Y. Okamura and F. Millett Biochemistry, 28, 6970 (1989).

The oxidation kinetics for tuna cytochrome c also reflected the presence of an electron transfer complex which is associated with submicrosecond kinetics (0.7 μs +/- 0.3 μs). This is surprising since the tuna cytochrome, like other mitochondrial c-cytochromes, contains several deletions which makes it structurally distinct from the bacterial cytochrome c_2[22]. The tuna cytochrome kinetics also exhibited a distinct, second bound component, characterized by an oxidation time of 12 μs.

The 0.7 ± 0.1 μs component of the R. capsulatus cytochrome c_2 also places it in this group of cytochromes which form an electron transfer complex exhibiting submicrosecond oxidation kinetics.

The c-cytochromes from horse, S. cerevisiae and C. krusei all showed the existence of bound components, but with markedly longer oxidation times of 19, 28 and 47 μs respectively.

The cytochromes c_2 from R. rubrum[9] and R. viridis did not exhibit a bound, reaction center-cytochrome electron transfer complex. Instead, the kinetics showed only a single, slower second order component.

Finally, the cytochrome c_{551} from P. aeruginosa, which is the smallest c-cytochrome and has the greatest exposure of the heme edge[22], showed no electron transfer to the reaction center on a time scale up to a millisecond.

The electron transfer from the c-cytochromes to the reaction center bacteriochlorophyll dimer is only weakly exothermic. The redox potential of the R. sphaeroides cytochrome c_2 is about 350 mV in solution[1,6,14] but shifts to approximately 300 mV when bound to the reaction center[6,14]. The redox potential of the bacteriochlorophyll dimer is 450 mV[1]. The lack of a correlation between the redox potentials of the c-cytochromes and the rates of electron transfer shows that these reactions are not limited by the thermodynamics, but are likely to be controlled by different configurations of the reaction center-cytochrome complexes. Although the cytochrome-reaction center association is known to be driven by electrostatic forces[1-6], there is no experimental information on the mechanisms of the electrostatic association.

We have begun to develop a detailed model of the molecular interactions responsible for the formation of the reaction center-cytochrome complexes. Electrostatic properties were calculated for the c-cytochromes whose crystal structures were available in the Brookhaven Protein Data Bank, as well as from a model of the R. sphaeroides cytochrome c_2 which was constructed from the closely homologous Paracoccus denitrificans cytochrome c_2[12]. Table II lists a comparison between the electron transfer efficiency with the reaction center and three electrostatic properties calculated from the crystal structures.

TABLE II Electrostatic properties of c-cytochromes

	net charge	dipole moment (debye)	average Coulomb potential (esu x 10^4)
sphaeroides	-1	640	9
tuna	7	305	52
capsulatus	1		
horse	7	310[a]	57
cerevisiae			
krusei	5		
rubrum	1	355	22
viridis	3		
aeruginosa	-3	190[b]	-15

[a]W.H. Koppenol and E. Margoliash, J. Biol. Chem., 257, 4426 (1982).

[b]D.W. Dixon, X. Hong and S.E. Woehler, Biophys. J., 56, 339 (1989).

Net charge is the most global electrostatic parameter of a protein. The cytochrome binding surface on the reaction center has a net charge of -7, which includes the amino terminal of the H-subunit and the carboxy terminals of the L and M subunits. The absence of a correlation between the net charge of the cytochromes and electron transfer efficiency shows that this is not a controlling parameter in formation of an electron transfer complex with the reaction center.

The dipole moment measures an inhomogeneous charge distribution. The ionic strength dependence of cytochrome c self exchange reactions have been found to be well described by the dipole properties of the c-cytochromes[15,23]. Similarly, the electron transfer activity of a series of horse cytochrome c derivatives with the ubiquinol-cytochrome c reductase and cytochrome c oxidase has also been shown to be related to the dipole moments of the cytochrome derivatives[16]. The absence of a correlation between cytochrome dipole moment and electron transfer capability suggests that the dipole moment is not a dominant factor in determining the configuration of the electron transfer complex with the reaction center.

The Coulomb potential provides a measure of local charge clustering. An average Coulomb potential was calculated for a patch above the exposed heme edge. Electron transfer is generally assumed to occur from the exposed heme edge, and electrostatic fields above the heme edge could play a role in determining the orientation of the cytochrome in the bimolecular electron transfer complex. Potentials at distances of 4 Å - 10 Å from

the heme CHC, C2C, C3C and CHD atoms were calculated along the lines connecting these atoms to the heme iron atom. The Coulomb potentials listed in Table II are the average of these values at a distance of 7 Å. Average Coulomb potential calculated at other distance showed proportional differences between the different cytochromes.

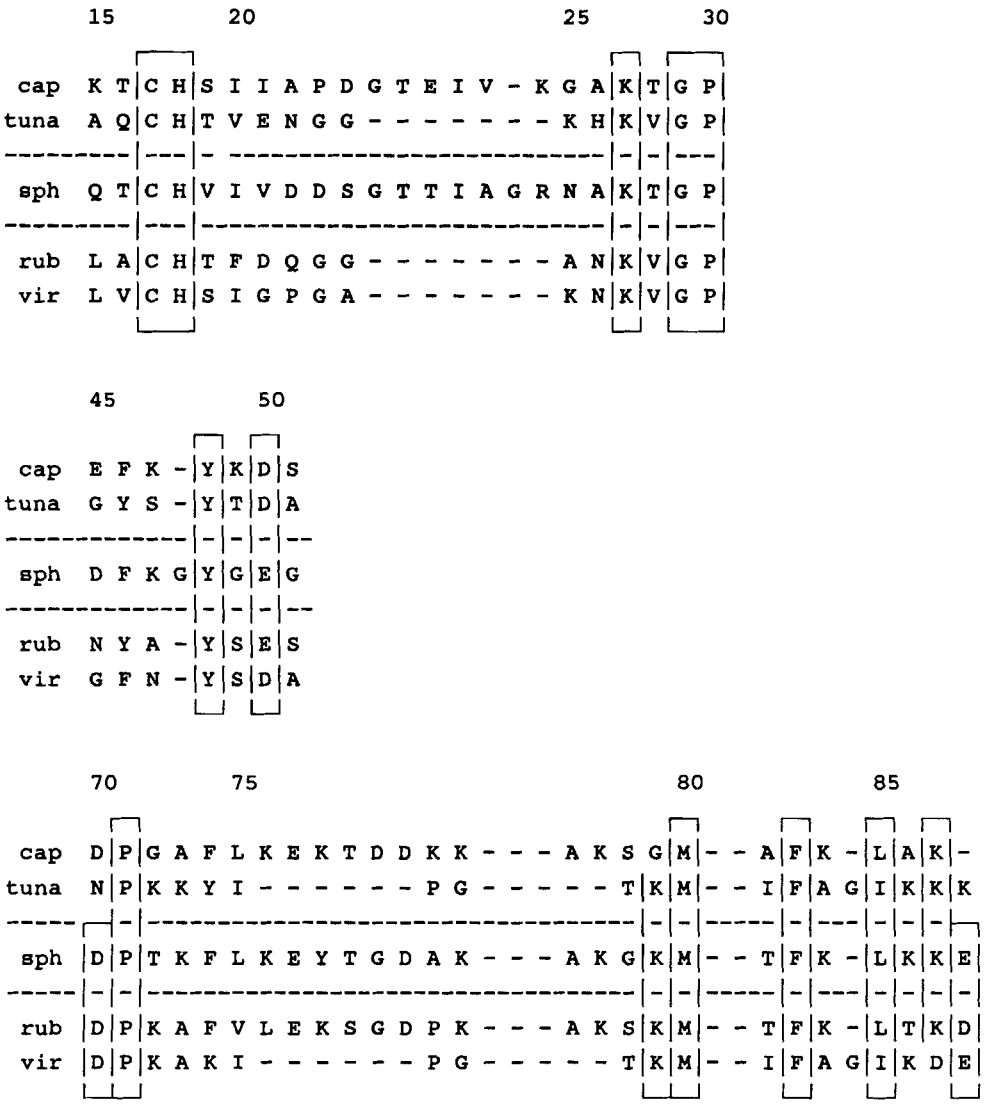
The comparisons in Table II suggest that the average Coulomb potential can provide a partial explanation for the observed electron transfer properties of reaction center-cytochrome complexes. The P. aeruginosa cytochrome is the only cytochrome which showed no capacity for electron transfer to the reaction center, and this is the only cytochrome with a net negative potential above the heme edge. This suggests that a positive potential is required for formation of an electron transfer complex, possibly in a "pre-orientation" step where the cytochrome rotates the surface with the exposed heme edge towards the reaction center as it approaches. The lack of a correlation with the electron transfer capacities of the other cytochromes suggests that other electrostatic or molecular interactions are required to define the final configuration of the complex.

Table III shows a comparison of amino acid sequences for the protein segments which surround the heme in five of the c-cytochromes. The sequence alignment is from Meyer and Kamen[22]. The sequence in the middle is from R. sphaeroides. The two on top are from R. capsulatus and tuna, both of which also formed reaction center-cytochrome complexes with submicrosecond electron transfer kinetics. The two sequences on the bottom are from R. rubrum and R. viridis, both of which were unable to form static electron transfer complexes with the reaction center. The boxes mark positions where either the top two or bottom two sequences matched the R. sphaeroides sequence. If the formation of an electron transfer complex with the reaction center required a specific amino acid sequence or required amino acid residues at specific positions, then the sequences on top should match the R. sphaeroides more closely than the sequences on the bottom. This is not seen, and both the electron transfer competent and incompetent cytochromes have equivalent overlaps with the R. sphaeroides sequence.

DISCUSSION

Several previous studies have examined how electrostatics control second order or steady-state electron transfer between pairs of redox proteins[16,24,25]. The flash-induced oxidation of the reaction center allows electron transfer with specific bound, reaction center-cytochrome c complexes to be observed and distinguished from second order diffusional reactions. We have characterized the electron transfer rates for a series of reaction center-cytochrome c complexes. A comparison to electrostatic properties leads to the following conclusions on role of electrostatics in the formation of these complexes.

TABLE III Amino acid sequences for protein segments surrounding the heme edge



1) A specific amino acid sequence or "lock and key" mechanism is not required for the formation of a reaction center-cytochrome c complex which is capable of submicrosecond electron. Instead, these results would suggest that there are likely multiple combinations of charge distributions which lead to the formation of functionally equivalent complexes.

2) A clustering of positively charged lysine residues around the heme edge is a necessary but not sufficient condition for formation of an efficient electron transfer complex with the reaction center. We suggest that a positive potential around the heme edge is likely to be required for initial alignment of the cytochrome and reaction center proteins, but that other molecular interactions besides a simple Coulomb potential above the heme edge is required for defining a configuration which permits submicrosecond electron transfer.

The characterization of electron transfer properties of a series of reaction center-cytochrome complexes will provide the experimental data needed to develop a more complete description of the molecular interactions responsible for the formation of these complexes.

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