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THE pH DEPENDENCE OF THE OXIDATION-REDUCTION MIDPOINT POTENTIAL OF CYTOCHROMES c_2 IN VIVO

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SUMMARY

A recent report by Pettigrew et al. [Biochim. Biophys. Acta 430, (1976), 197–208] has examined the pH dependence of the oxidation-reduction midpoint potential of cytochromes c_2 in vitro. In media of low ionic strength, these workers identified several p*K*s on the oxidized forms of the cytochromes, and in some cases there were also p*K*s on the reduced species. In this work we examine the pH dependence of the midpoint potentials of the cytochromes in situ, attached to the chromatophore membrane. Under these conditions no p*K* values are detected, and we conclude that in vivo there is no net change in the protonation of cytochrome c_2 during oxidation or reduction.

INTRODUCTION

Cytochromes c_2 are a family of proteins found in most photosynthetic non-sulfur bacteria (Rhodospirillaceae). When the bacteria are grown photosynthetically, cytochrome c_2 usually functions as the immediate electron donor to the oxidized bacteriochlorophyll of the reaction center (eg. ref. 1). In vivo the cytochrome is found in the periplasmic space between the cell membrane and the cell wall, and when chromatophores are prepared, the cytochrome is trapped inside the vesicles [2]. In *Rhodopseudomonas sphaeroides* it has been shown that there are two cytochrome c_2 molecules associated with each reaction center [1], and that between pH 6 and 9 the oxidation-reduction midpoint potential (E_m) value of the cytochrome complement is practically independent of pH [1], so that the cytochrome could not act as a redox-coupled hydrogen carrier.

A recent report of Pettigrew et al. [3] has examined the equilibrium determined oxidation-reduction midpoint potentials of isolated buffer-soluble cytochromes c_2 from *Rps. sphaeroides*, *Rps. capsulata* and *Rhodomicrobium vannielii*. In dilute solution, they found that the midpoint potentials varied with pH over at least part of the pH range between pH 4 and 11, and they identified p*K*s on the oxidized forms of all three cytochromes; at pH 9.4 in *Rps. capsulata*, at pH 6.1 and 8.0 in *Rps. sphaeroides*, and at pH 6.3 and 9.4 in *Rm. vannielii*. There were also p*K*s on the reduced forms of

the *Rps. sphaeroides* (at pH 6.4) and *Rm. vannielii* (at pH 7.4) cytochromes. These are potentially very important findings, because they could imply that under certain conditions the cytochromes might act as redox coupled hydrogen carriers. This has prompted us to re-examine the in vivo E_m/pH dependency of *Rps. sphaeroides* cytochrome c_2 over a wider pH-range, examining the two molecules of the cytochrome complement individually. We have also extended our investigations to include c_2 *Rps. capsulata* (see ref. 4).

Our results indicate that in chromatophores of both *Rps. sphaeroides* and *Rps. capsulata*, the midpoint potential of cytochrome c_2 is effectively independent of pH between pH 5 and 11, and that throughout this range of pH there are two thermodynamically and kinetically equivalent cytochrome c_2 molecules which are capable of donating electrons to the reaction center.

METHODS

Rps. sphaeroides Ga and *Rps. capsulata* BY 761 (derived from *Rps. capsulata* B 10), which are both green mutants, were grown anaerobically in the light with succinate as sole carbon source as described previously [1]. Chromatophores were prepared, using a French pressure cell, in a medium of 100 mM KCl, 20 mM *N*-morpholino propane sulfonic acid, 1 mM $MgCl_2$ pH 7.2 [2]. The effective ionic strength of this medium is approximately 114 mM, and when buffers at other pH values were used, their concentrations were adjusted to give an approximately equal ionic strength.

Redox potentiometry was carried out as previously described [1], using 7 μM *N,N,N',N'*-tetramethylphenylenediamine as a redox mediator. Flash activation was provided by a xenon flash lamp (pulse width at half height = 6 μs) in a rapidly responding dual wavelength spectrophotometer [1].

RESULTS

Figs. 1 and 2 show representative flash-activated redox titrations of cytochrome c_2 in both *Rps. sphaeroides* and *Rps. capsulata*. The extent of cytochrome oxidized by the first and the second near-saturating single turn-over flashes* (labelled 1st and 2nd c_2) is plotted as a function of redox potential. The lines drawn through the points reflect the expected lines if two identical cytochrome hemes shared each reaction center. In this case the amount of cytochrome oxidized on the first flash will follow the curve $2x-x^2$, where x is the proportion of reduced cytochrome. The amount oxidized by the second flash will then follow the line of x^2 (see refs. 1, 5). In both species the data fits such curves very well, at all values of pH.

Fig. 3 shows the midpoint potentials of the cytochromes, determined in experiments similar to those shown in Figs. 1 and 2, over the pH range pH 5–11. In *Rps. sphaeroides* some experiments have also used equilibrium dark titrations where the oxidation or reduction of the cytochrome was monitored simply as the absorption at 550–540 nm as a function of redox potential [1]. The data for *Rps. capsulata* are in

* In fact some 15 % of the cytochrome c_2 was oxidized on the third flash (see refs. 1, 5) and we have included this with the amount oxidized by the second flash as the "2nd c_2" in Figs. 1 and 2.

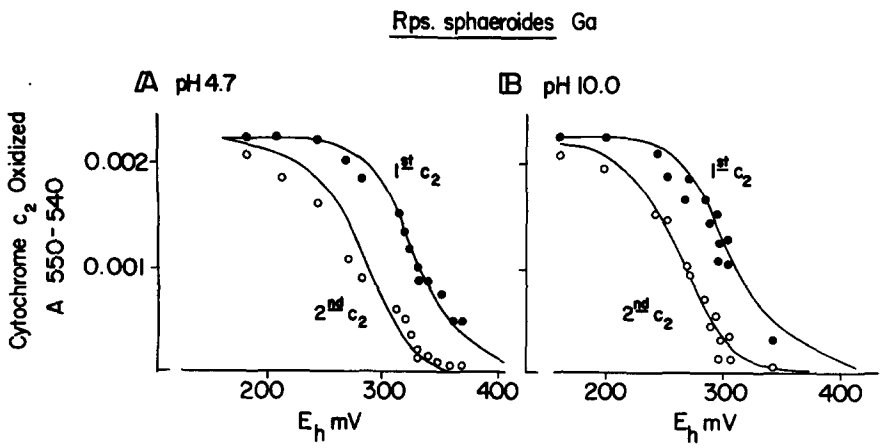

Fig. 1. Redox titrations of cytochrome c_2 in *Rps. sphaeroides*. Chromatophores (bacteriochlorophyll = 20.8 μM) were suspended in 100 mM KCl, 20 mM buffer (*N*-morpholino ethane sulfonic acid at pH 4.7, glycine at pH 10).

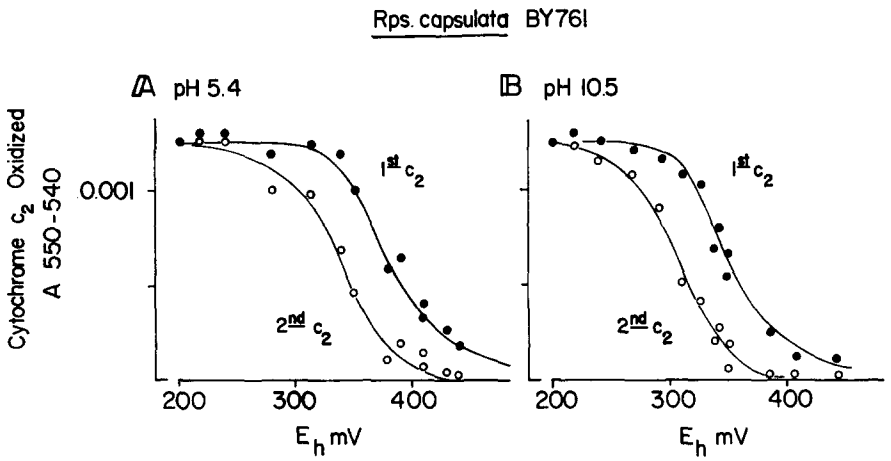

Fig. 2. Redox titrations of cytochrome c_2 in *Rps. capsulata*. Chromatophores (bacteriochlorophyll = 18.2 μM) were suspended in similar buffers to those used in Fig. 1.

good agreement with those of Evans and Crofts [4] although these authors reported somewhat higher midpoint potentials in some experiments at pH values more acid than pH 7. We did not observe this phenomenon. Fig. 3 shows that the E_m of cytochrome c_2 in vivo at pH 7.0 is +300 mV in *Rps. sphaeroides* and +350 mV in *Rps. capsulata*. These compare with the in vitro values of Pettigrew et al. [3] of +350 mV for *Rps. sphaeroides* and +370 mV for *Rps. capsulata*.

DISCUSSION

The pH dependency of the midpoint potential of cytochrome c_2 in vivo

In both *Rps. sphaeroides* and *Rps. capsulata*, the midpoint potential of cyto-

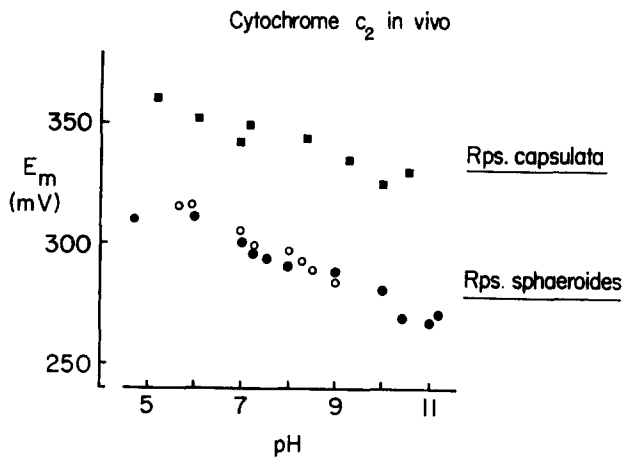

Fig. 3. The pH dependency of the midpoint potential of cytochrome c_2 in vivo. The solid symbols represent data obtained as in Figs. 1 and 2 except that appropriate pH buffers were used. The open symbols for *Rps. sphaeroides* are taken from reference 1.

chrome c_2 in vivo varied with pH by less than -10 mV/pH unit throughout the pH range pH 5–11. This implies that there is no net change in the protonation of the cytochrome during its equilibrium redox reactions. These experiments do not rule out the possibility that protons are involved in the dynamic, functional redox reactions, but so far there is no evidence to suggest such an involvement. Taken at their face value, the experiments indicate that cytochrome c_2 does not act as a hydrogen carrier in vivo. A similar lack of pH dependency, and cytochrome : reaction center stoichiometry of 2, has also been seen with the cytochrome c_2 of *Rhodospirillum rubrum* (Prince, R.C., unpublished observation) and the functionally similar cytochromes *c*-555 of *Chromatium vinosum* [5] and cytochromes *c*-558 of *Rps. viridis* [6].

The reasons for the different thermodynamic properties of cytochrome c_2 when in dilute solution [3] or attached to the chromatophore membrane (this work) are not clear, but both the E_m values and the pK values appear altered under the different conditions. A similar shift in the E_m of mammalian cytochrome *c* occurs upon binding to various mitochondrial [7] and liposome [8] membranes, from about $+280$ mV to about $+230$ mV at pH 7.0, although the E_m of the buffer soluble cytochrome is dependent on the ionic strength [9] and ionic composition [7] of the solution. Rodkey and Ball [10] demonstrated that mammalian cytochrome *c* has pKs at pH 7.8 (oxidized form) and pH 1.75 (reduced form) when in a soluble form, but whether these occur at similar values in the membrane bound state is an open question, since E_m/pH determinations have not yet been extensively investigated.

The simple explanation of the E_m shift of mammalian cytochrome *c* was that it represented a preferential binding of the oxidized form to membrane components, and direct binding studies [11] supported this. It is possible from the work presented here that the thermodynamic alterations which occur on binding to the membrane may be more extensive, with binding differences between protonated and unprotonated oxidized and reduced forms. If this applies to the cytochromes c_2, the membrane appears to have a higher affinity for the protonated oxidized form than the un-

protonated oxidized species, which has the effect of shifting the oxidized pK to more alkaline (beyond pH 10.5) values. This would ensure that in the physiological pH-range there was no net change of protonation upon oxidation or reduction of the cytochrome.

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