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pH DEPENDENCE OF THE OXIDATION-REDUCTION POTENTIAL OF CYTOCHROME c_2

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SUMMARY

The pH dependence of the spectra and of the oxidation-reduction potential of three cytochromes c_2, from *Rhodopseudomonas capsulata*, *Rhodopseudomonas sphaeroides* and *Rhodomicrobium vannielii*, were studied. A single alkaline pK was observed for the spectral changes in all three ferricytochromes. In *Rps. capsulata* cytochrome c_2 this spectroscopic pK corresponds to the pK observed in the dependence of oxidation-reduction potential on pH. For the other two cytochromes the oxidation-reduction potential showed a complex dependency on pH which can be fitted to theoretical curves involving three ionizations. The third ionization corresponds to the ionization observed in the spectroscopic studies but the first two occur without changes in the visible spectra.

The possible structural bases for these ionizations are discussed.

INTRODUCTION

The cytochromes c_2 are a family of proteins found in most photosynthetic non-sulphur purple bacteria (the *Rhodospirilliaceae*). The most thoroughly studied protein (that from *Rhodospirillum rubrum*) is related to the mitochondrial cytochromes c in both sequence [1] and three-dimensional structure [2]. The oxidation-reduction potentials of many of the cytochromes c_2 have been measured (reviewed in ref. 3) but the data are scattered, incomplete and sometimes inconsistent and the present survey was undertaken to examine the different cytochromes under the same experimental conditions.

A program of sequence determination for these proteins is under way [4, 5] and this may provide an excellent opportunity for studying variation in properties with changes in structure. This paper reports a detailed analysis of the pH dependence of the oxidation-reduction potential for three cytochromes c_2 from *Rhodopseudomonas*

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capsulata, *Rhodopseudomonas sphaeroides* and *Rhodomicrobium vannielii*. In the comparative study of Kamen and Vernon [6] *Rps. capsulata* cytochrome c_2 was found to have a relatively constant mid-point potential of 320–330 mV between pH 5 and pH 10. The results of Evans and Crofts [7] also showed a lack of pH dependence and a potential of 340 mV whether the cytochrome was present in solution or attached to chromatophores. *Rps. sphaeroides* cytochrome c_2 was shown to have a mid-point potential of 340 mV at pH 7 [8] but Dutton et al. [9] found that in chromatophores $E_{\mathrm{m,\ pH7}}$ was 295 mV. The purified cytochrome in water solution had a mid-point potential of 345 mV which was relatively constant between pH 6 and 8. $E_{\mathrm{m,\ pH7}}$ for partially purified *Rm. vannielii* cytochrome c_2 was reported to be 304 mV [10].

METHODS

Rhodomicrobium vannielii strain 3.1.1 (ATCC 17100), *Rhodopseudomonas sphaeroides* strain 2.4.1 (ATCC 17023) and *Rhodopseudomonas capsulata* strain Saint Louis (ATCC 23782) were grown anaerobically in the light on a complex medium [11] and the cytochromes c_2 were isolated using standard methods [12].

Spectra were recorded on a Cary 14 or an Aminco DW2 spectrophotometer. The method for titration of the 695 nm band is described in the legend to Fig. 4. Reverse titrations were not normally performed because of local precipitation of cytochrome on addition of acid, but reversibility was checked by passing the alkaline cytochrome solution through a Sephadex G-25 column and estimating the recovery of the 695 nm absorption.

The oxidation-reduction potential at a particular pH was measured by the method of mixtures [13, 14]. A solution of cytochrome in buffer was completely oxidized by addition of a known amount of ferricyanide and the spectrum recorded at the α-peak. A known amount of ferrocyanide was then added and the position of the redox equilibrium was estimated spectroscopically. The pH of the solution was measured and then sodium dithionite was added to obtain the fully reduced spectrum. The mid-point potential (E_{m}) of the cytochrome was calculated assuming $n = 1$ and using the data of Hanania et al. [15] to calculate the potential of the solution (E_{h}) for a particular ionic strength. Experimental details are described in the legend to Fig. 1.

E_{h} was set at the desired level near the E_{m} for the cytochrome. For much of the pH range in all three studies the solution contained 0.5 mM potassium ferrocyanide and 0.17 mM potassium ferricyanide. The total ionic strength was 0.006–0.008 mol/l and the solution potential was 347–349 mV. Under these conditions of low ionic strength the mid-point potentials of the cytochromes and the ferro-ferricyanide couple are similar, so that nearly equimolar solutions of the latter may be used.

The potential of the ferri-ferrocyanide couple is not constant with pH below pH 5. For the results between pH 4 and pH 5 a small correction (in the range of +5 mV at pH 4.2 to +1 mV at pH 4.8) was therefore made to the value of E'_0 (ferro-ferricyanide) based on the data of Fig. 3 of Hanania et al. [15].

In the alkaline pH region a potential of 287 mV was obtained with 0.5 mM potassium ferrocyanide and 0.017 mM potassium ferricyanide. At these low concentrations of ferricyanide the additional amount produced during reduction of the ferricytochrome must be calculated and taken into account. For this purpose, difference extinction coefficients $\Delta\varepsilon_{\alpha\text{-peak (reduced-oxidized)}}$ were measured relative to the extinction of the pyridine ferrohemochrome at 550 nm (29.1 $mM^{-1} \cdot cm^{-1}$, ref. 16). The values obtained were 19.8 $mM^{-1} \cdot cm^{-1}$ for *Rm. vannielii* cytochrome c_2, 20.7 $mM^{-1} \cdot cm^{-1}$ for *Rps. sphaeroides* cytochrome c_2 and 20.6 $mM^{-1} \cdot cm^{-1}$ for *Rps. capsulata* cytochrome c_2.

Potassium ferro- and ferricyanide solutions were prepared fresh daily. They were checked at the beginning and end of a series of experiments by measuring the E_m for horse cytochrome *c* (Sigma type VI).

The method of mixtures, being simple and rapid, allows collection of a large amount of data at different pH values. The validity of the method as applied to the cytochromes c_2 was established in three ways.

(1) Oxidative titrations were performed to determine the value of *n* at three pH values. Because of the variation of the ferro-ferricyanide potential with ionic strength [15] titrations by the method of mixtures could give anomalous results if the contribution of the titrant to the ionic strength was appreciable. Therefore the potential of the solution was calculated for each addition of ferricyanide, taking into account the change in ionic strength. If solution potential (E_h) is plotted against log (Cyt (red)/ Cyt (ox)), theoretical slopes of 60 mV for $n = 1$ and 30 mV for $n = 2$ are predicted.

(2) Some determinations were made under anaerobic conditions in a Thunberg cuvette. A cycle of evacuation and flushing with argon was repeated five times before ferrocyanide was added to establish an equilibrium.

(3) The potentials of some solutions were measured directly with a combination platinum-calomel electrode (Ingold, cat. no. 15023). As shown in Figs. 1–3 the results of the different methods were in good agreement.

Small inaccuracies may be introduced into the calculated potentials for the following reasons.

(a) Equimolar solutions of ferro- and ferricyanide were not used and the ratio was not constant throughout the pH range. Thus the relationship between potential and ionic strength established by Kolthoff and Tomsicek [17] and Hanania et al. [15] was not strictly applicable. The error involved was small, however, as solutions of the same pH but containing different ratios of ferri- and ferrocyanide gave closely similar results for $E_{m\,(\text{cytochrome})}$.

(b) O'Reilly [18] showed that the potential of ferro-ferricyanide solutions was dependent on the type of buffer present. Under our conditions this error was small as solutions of different buffers at the same pH gave very similar potentials with the combination electrode.

RESULTS

Table I shows the mid-point oxidation-reduction potentials of the three cytochromes at different pH values.

Rps. capsulata cytochrome c_2

Over the pH range examined, the curve of E_m versus pH (Fig. 1) can be fitted to the simple equation

$$E_m = \tilde{E} + \frac{RT}{nF} \ln \frac{H^+}{H^+ + K} \qquad (1)$$

TABLE I

OXIDATION-REDUCTION POTENTIALS OF CYTOCHROMES c_2

Most values of E_m were determined by the aerobic single point method described in the text and in the legend of Fig. 1.

Rps. capsulata		*Rm. vannielii*		*Rps. sphaeroides*	
pH	E_m	pH	E_m	pH	E_m
4.21	370	4.65	392	4.28	368
4.61	371	4.65	393*	4.58	369
5.28	368**	4.8	388**	4.95	367***
5.44	371	5.0	391	5.0	368**
5.71	370	5.3	392***	5.1	368*
6.18	368	5.3	393	5.13	366
6.41	367	5.4	389	5.74	364
6.77	365	5.6	394	6.18	358
6.9	368***	5.75	389	6.66	355
7.17	368	5.95	387	6.9	350**
8.05	366	6.15	383	6.99	352***
8.42	366	6.3	379	7.16	350
8.86	360	6.7	369	7.2	353*
9.06	355	6.9	357*	7.5	347
9.42	348	6.9	356***	7.73	341
9.63	344	7.0	356	7.98	334
9.65	340***	7.4	344	8.3	321
9.77	339**	7.6	341	8.35	333*
10.3	313	7.9	335	8.6	308
10.75	291	8.0	334**	8.65	300**
		8.2	333	8.77	299
		8.35	331*	9.05	289
		8.45	331	9.25	279
		8.6	328	9.27	271***
		8.75	327	9.35	282 *
		8.9	326	9.51	265
		9.15	318	9.76	249
		9.4	311		
		9.4	313***		
		9.6	304		
		9.95	286*		
		10.0	289		
		10.0	288**		
		10.25	277		
		10.5	253		

* E_h was measured directly with a combination electrode.

** E_m was determined under anaerobic conditions.

*** E_m was determined from a complete titration.

K is an apparent equilibrium constant for the heme-linked dissociation of a proton in the ferricytochrome. As indicated in Fig. 1, the pK is 9.4. $\tilde{E}$ was derived from extrapolation of E_m to pH 0 along a line of constant slope [19] and is 368 mV.

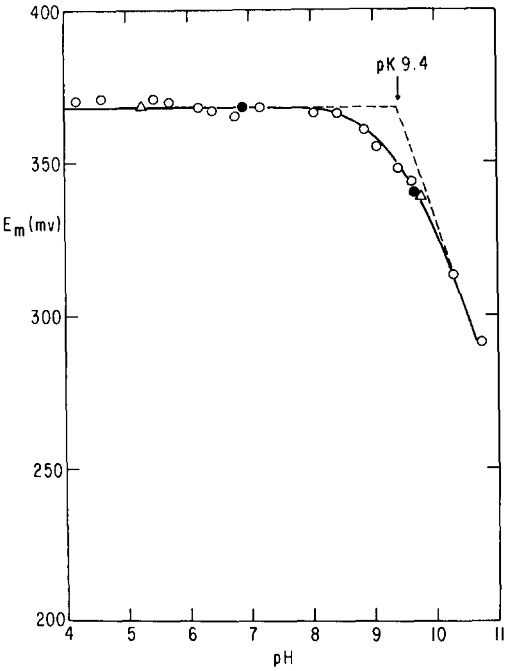

Fig. 1. pH dependence of the oxidation-reduction potential of *Rps. capsulata* cytochrome c_2. (○) Single equilibrium measurement in air; (●) titration results; (△) anaerobic single equilibrium results. Ionic strength 0.006–0.008 mol/l, $T = 25$ °C, cytochrome concentration $= 5 \cdot 10^{-6}$ M. Potassium ferrocyanide was 0.5 mM, potassium ferricyanide was 0.017 or 0.17 mM. Buffers were 1 mM acetic acid/sodium acetate (pH 4–5.8); 1 mM NaH_2PO_4/Na_2HPO_4 (pH 5.8–7.5); 1 mM Tris · HCl (pH 7.5–8.8); 1 mM glycine/NaOH (pH 8.8–10.5). pH was measured before addition of dithionite. Solid line, theoretical curve according to Eqn. 1.

The titration results for the effect of pH on the 695 nm absorption band are shown in Fig. 4A. The pattern is clearly complex but 80 % of the absorption change could be fitted to a theoretical curve with a pK of 9.05. The remainder of the band disappeared between pH 10.5 and pH 11.5 accompanied by large changes at 525 nm with a shift to 533 nm and a broad increase in absorption between 530 nm and 600 nm.

Rm. vannielii cytochrome c_2

The oxidation-reduction potential showed a pronounced pH dependence in the neutral pH region (Fig. 2). Using the approach of Clark [19] it is evident that there are at least three ionizations which affect the oxidation-reduction potential in the pH region studied. The changes in slope define these as two apparent ionizations in the oxidized form with pK values of approximately 6(pK_{o1}) and 9(pK_{o2}) and one in the reduced form with a pK near 8 (pK_r).

These ionizations can be represented as follows:

$$H_2ox = Hox^- + H^+ \qquad K_{o1} = \frac{(Hox^-)(H^+)}{(H_2ox)}$$

$$\mathrm{Hox}^- = \mathrm{ox}^{2-} + \mathrm{H}^+ \qquad K_{o2} = \frac{(\mathrm{ox}^{2-})(\mathrm{H}^+)}{(\mathrm{Hox}^-)}$$

$$\mathrm{H_2red} = \mathrm{Hred}^- + \mathrm{H}^+ \qquad K_r = \frac{(\mathrm{Hred}^-)(\mathrm{H}^+)}{(\mathrm{H_2red})}$$

At pH values below pK_{o1} Eqns. 2 and 3 apply.

$$\mathrm{H_2ox} + e = \mathrm{H_2red} \tag{2}$$

$$E_m = \tilde{E} + \frac{RT}{nF} \ln \frac{\mathrm{H_2ox}}{\mathrm{H_2red}} \tag{3}$$

From these orienting relationships the procedures of Clark [19] can be used to derive Eqn. 4.

$$E_m = \tilde{E} + \frac{RT}{nF} \ln \frac{(\mathrm{H}^+)^2 + K_r(\mathrm{H}^+)}{(\mathrm{H}^+)^2 + K_{o1}(\mathrm{H}^+) + K_{o1}K_{o2}} \tag{4}$$

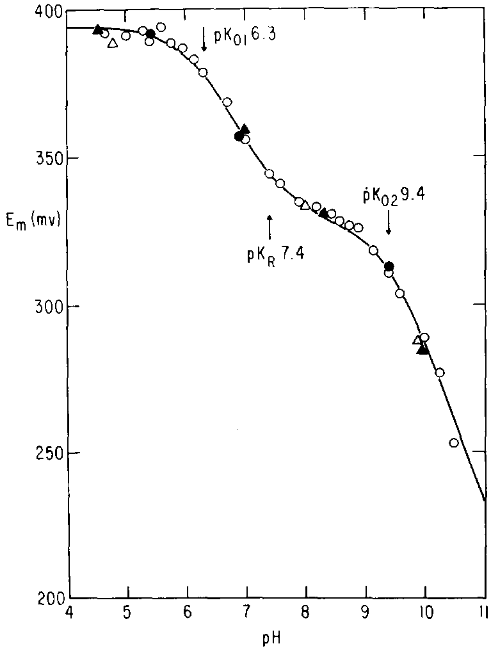

Fig. 2. pH dependence of the oxidation-reduction potential of *Rm. vannielii* cytochrome c_2. (○) Single equilibrium measurement in air; (●) titration results; (△) anaerobic single equilibrium results; (▲) potentials measured directly with a platinum-calomel electrode. Ionic strength 0.006–0.008 mol/l, $T = 25$ °C, cytochrome concentration $= 5 \cdot 10^{-6}$ M. Potassium ferrocyanide was 0.5 mM, potassium ferricyanide was 0.017 or 0.17 mM. Buffers were 1 mM acetic acid/sodium acetate (pH 4–5.8); 1 mM NaH_2PO_4/Na_2HPO_4 (pH 5.8–7.5); 1 mM Tris · HCl (pH 7.5–8.8); 1 mM glycine/NaOH (pH 8.8–10.5). pH was measured before addition of dithionite. Solid line, theoretical curve according to Eqn. 4.

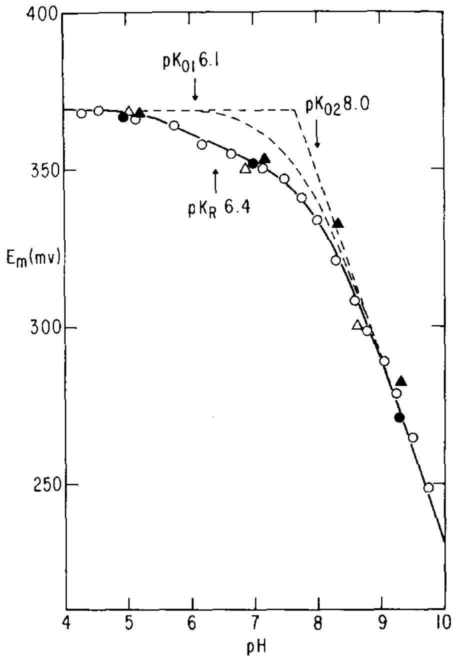

Fig. 3. pH dependence of the oxidation-reduction potential of *Rps. sphaeroides* cytochrome c_2. (○) Single equilibrium measurement in air; (●) titration results; (△) anaerobic single equilibrium results; (▲) potentials measured directly with a platinum-calomel electrode. Ionic strength 0.006–0.008 mol/l, $T = 25$ °C, cytochrome concentration $= 5 \cdot 10^{-6}$ M. Potassium ferrocyanide was 0.5 mM, potassium ferricyanide was 0.017 or 0.17 mM. Buffers were 1 mM acetic acid/sodium acetate (pH 4–5.8); 1 mM NaH_2PO_4/Na_2HPO_4 (pH 5.8–7.5); 1 mM Tris · HCl (pH 7.5–8.8); 1 mM glycine/NaOH (pH 8.8–10.5). pH was measured before addition of dithionite. Solid line, theoretical curve according to Eqn. 4. Broken curve, single ionization, $pK = 7.65$, $\tilde{E} = 369$ mV.

Values of the apparent ionization constants were selected empirically to give a theoretical curve (Fig. 2) which best fitted the experimental results with $pK_{o1} = 6.3$, $pK_r = 7.4$, $pK_{o2} = 9.4$ and $\tilde{E} = 394$ mV.

The results of titration of the 695 nm band are shown in Fig. 4B. Unlike the results for the oxidation-reduction potential, this change with pH could be fitted to a theoretical curve for a single ionization with a pK of 9.1.

Rps. sphaeroides cytochrome c_2

This cytochrome appeared to show a pH dependence of oxidation-reduction potential intermediate between the above two cases. The data of Fig. 3 clearly did not correspond to a single ionization process, but pK values were more difficult to assign than in the case of *Rm. vannielii* cytochrome c_2. Using Eqn. 4, a curve was fitted by trial and error with $pK_{o1} = 6.1$, $pK_r = 6.4$, $pK_{o2} = 8.0$ and $\tilde{E} = 369$ mV.

The results of the titration of the 695 nm absorption band could be fitted to a theoretical curve for a single ionization with a pK of 7.9 (Fig. 4C).

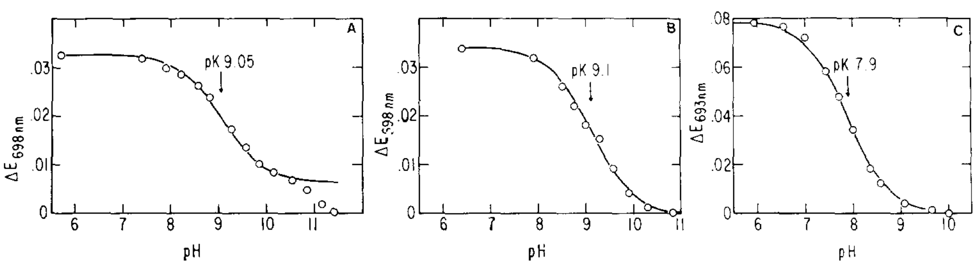

Fig. 4. Titration of the 695 nm absorption band of (A) *Rps. capsulata* cytochrome c_2, (B) *Rm. vannielii* cytochrome c_2 and (C) *Rps. sphaeroides* cytochrome c_2. The pH of an unbuffered solution of ferricytochrome was adjusted with 0.1 M NaOH and the spectrum in the region of the 695 nm band was recorded after each addition. Cytochrome concentration was approximately 10^{-4} M and the titration was performed in the presence of $3 \cdot 10^{-4}$ M potassium ferricyanide. The solid lines are theoretical curves for the Hill equation, pH = pK+log ([alkaline species]/[neutral species]). pK values for the theoretical curves are shown.

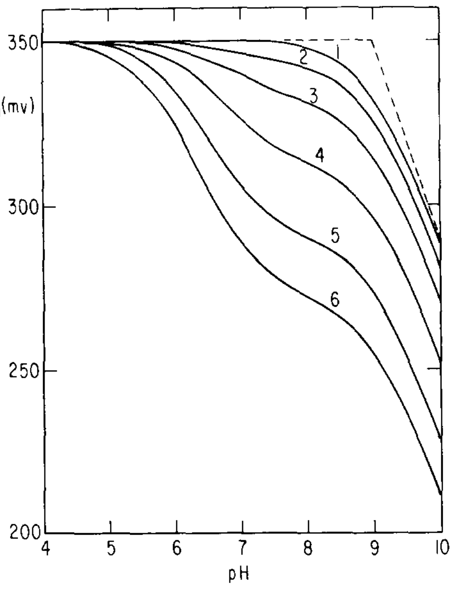

Fig. 5. Theoretical curves for three ionizations affecting the oxidation-reduction potential. Curves shown are from Eqn. 4 in the text. K_r and K_{02} were held constant at 10^{-7} M and 10^{-9} M, respectively, while K_{01} was varied between $2 \cdot 10^{-6}$ M and 10^{-7} M. Values of K_{01} are (1) 10^{-7} M ($= K_r$), (2) $1.3 \cdot 10^{-7}$ M, (3) $2 \cdot 10^{-7}$ M, (4) $4 \cdot 10^{-7}$ M, (5) 10^{-6} M and (6) $2 \cdot 10^{-6}$ M. The curve of (1) is identical to that of a single ionization with pK = pK_{02} = 9.

DISCUSSION

Interpretation of Eqn. 4

Clark [19] established that features of the curve of E_m versus pH for oxidation-reduction systems could be interpreted as due to ionizations of the oxidized and reduced species. A change in the slope of such curves to more negative values indicated a pK in the oxidized form while a change to more positive values indicated a pK in the reduced form.

Although proteins are more complex molecules than those originally considered by Clark, his methods have been used in certain cases to describe successfully their behavior (refs. 20, 21 and Appendix to ref. 19). The simplest interpretation of the E_m versus pH curves for *Rps. sphaeroides* and *Rm. vannielii* cytochromes c_2 is that they involve three ionizations, the first and last in the oxidized species and the second in the reduced. Kakuno et al. [22] interpreted their results with *Rsp. rubrum* cytochrome c_2 in the same way.

Using Clark's procedures, Eqn. 4 can be derived which includes three ionization constants. The different shapes of the curves in Figs. 2 and 3 are due to different relative positions of the apparent pK values. In Fig. 5 the effect of varying K_{o1} in Eqn. 4 is shown; as K_{o1} approaches K_r the curve changes from one like the experimental curve of *Rm. vannielii* cytochrome c_2 to one like that of *Rps. sphaeroides* cytochrome c_2 and finally when $K_{o1} = K_r$ a curve for a single ionization with pK_{o2} is obtained. Thus the simple pattern shown by *Rps. capsulata* cytochrome c_2 may be an example of the limiting case where pK_{o1} = pK_r.

It is possible that pK_{o1} and pK_r are ionizations of the same group in the oxidized and reduced forms of the proteins. The extent of separation of these two apparent pK values may depend on the proximity of this group to the heme. Because of the net charge of +1 on the oxidized heme and 0 on the reduced, a lowered pK might be expected from electrostatic considerations for a heme-linked ionization in the oxidized species. According to this scheme, the three cytochromes c_2 may have an ionizable group in common which is closest to the heme in *Rm. vannielii* cytochrome c_2 and so distant in the case of *Rsp. capsulata* cytochrome c_2 that the loss of the proton is unaffected by the charge on the oxidized heme.

Because the heme environment provided by the protein is complex, the preceding explanation based on Clark's analysis may not be the only possible interpretation of the changes in oxidation-reduction potential with pH. A remote ionizing group may affect the heme indirectly through a conformational change in the molecule. Such a change could alter the position of a charged or hydrophobic group in the vicinity of the heme or involve extensive rearrangement of the peptide chain with resultant changes in dielectric [23, 24]. It is probable that large changes in protein conformation would be reflected in changes in the spectra. The correlation between spectroscopically observed ionizations and those observed in the E_m versus pH curves is discussed below.

Apparent ionizations detected in the spectroscopic titrations

pK_{o1} and pK_r for *Rps. sphaeroides* and *Rm. vannielii* cytochromes c_2 are not associated with changes in the spectra of the proteins. This implies that these ionizations do not affect the primary ligand field of the iron. In contrast, the apparent pK for the loss of the 695 nm absorption band is similar to pK_{o2} in all three cytochromes c_2. This type of spectral transition has been extensively studied in the case of horse cytochrome *c* but its structural basis is still a matter of conjecture [25–27]. Davis et al. [25] have shown that an ionization with a pK of 11 followed by a conformational equilibrium favoring the alkaline species is involved and results in an observed pK of 9 in the spectra and the oxidation-reduction potential. The 695 nm absorption band has been associated with methionine coordination [28, 29] but it is not known whether the disappearance of the band is due to a substitution of this residue or to a more subtle change in the ligand field as a result of the conformational change.

Thus in the case of the cytochromes c_2 the spectral transition may have a similar complex structural basis and the observed stabilization of the oxidized form relative to the reduced (pK_{o2}) may not be interpretable in the simple terms of loss of a proton near the oxidized heme.

The group involved in the ionization with pK_{o2} in the oxidized form must also ionize in the reduced cytochrome. No further changes are observed, however, in the E_m versus pH curves. This may mean that the pK values in the oxidized and reduced forms have been so separated that the pK in the reduced form occurs outside the pH range studied, or that the ionization of the group in the reduced molecule does not affect the heme environment.

In the preceding discussion we have considered the possibility that an ionization gives rise to a conformational change in the protein which affects the oxidation-reduction potential. A second means whereby an ionization remote from the heme may influence the heme environment is through a hydrogen bonding network of the type proposed by Salemme et al. [2] for *Rsp. rubrum* cytochrome c_2 on the basis of the X-ray crystallographic structure. In this case the ferricytochrome is stabilized by the net negative charge of the internal tyrosine residue 70 which is part of a hydrogen bond network involving tyrosine 52 and serine 89. Protonation of this network at serine 89 would be expected to destabilize the oxidized state and result in a higher oxidation-reduction potential. However, the hydrogen-bonded network found in *Rsp. rubrum* cytochrome c_2 is not present in any of the cytochromes c_2 studied here. None of the three has tyrosine 52 nor an alternative hydrogen-bonding residue in this position [4, 5]. *Rps. sphaeroides* cytochrome c_2 has glycine in place of serine 89. It is possible that other residues may form a similar network in these three proteins but a system of the form found in *Rsp. rubrum* cytochrome c_2 cannot be the structural basis for the pH dependence of the oxidation-reduction potentials observed in this study.

Amino acid sequences and possible ionizing groups

The sequences of the three cytochromes c_2 have been determined [4, 5]. Rather than suggesting candidates for the ionizing groups involved, the primary structures tend to eliminate possibilities. The absence of a conserved hydrogen bond network maintaining charge in the interior of the molecule has been discussed. Histidine is an obvious example of a side chain which ionizes at neutral pH values, yet *Rm. vannielii* cytochrome c_2 has only one histidine and this presumably forms the fifth iron ligand [2, 5] and is therefore un-ionized throughout the pH range studied. *Rps. capsulata* cytochrome c_2 is unique among the cytochromes c_2 [4] and mitochondrial cytochromes c [30] in lacking a lysine residue immediately before the methionine, which forms the sixth iron ligand. This lysine has been proposed as the most likely replacement for methionine at alkaline pH [29, 31, 32]. However, the alkaline transitions of both the spectrum and the oxidation-reduction potential of *Rps. capsulata* cytochrome c_2 are very similar to those of horse cytochrome c.

The oxidation-reduction potentials of the cytochromes c_2 and the mitochondrial cytochromes c

The oxidation-reduction potentials of the mitochondrial cytochromes *c* studied are closely grouped between 243 and 265 mV [33, 34]. The published values for the cytochromes c_2 (reviewed in ref. 3) form a broader but non-overlapping range between 290 and 400 mV. Thus the oxidation-reduction potential of *Rm. vannielii* cytochrome c_2 falls within the cytochrome c_2 class. Ambler et al. [5] have shown that the sequence of *Rm. vannielii* cytochrome c_2 shows as much overall similarity to the mitochondrial cytochromes *c* as it does to other cytochromes c_2. A continuum of structural change between the two groups may eventually be demonstrated. This is not, however, reflected in certain properties for, in addition to the distinctively high oxidation-reduction potential, *Rm. vannielii* cytochrome c_2 resembles the other cytochromes c_2 in its poor reaction with bovine cytochrome *c* oxidase (ref. 35 and Errede, B., personal communication).

Thus in these two respects the families of cytochrome c_2 and mitochondrial cytochrome *c* are distinct and this separation presumably has a subtle structural basis not evident in the overall sequence comparisons.

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REFERENCES

1 Dus, K., Sletten, K. and Kamen, M. D. (1968) J. Biol. Chem. 243, 5507–5518
2 Salemme, F. R., Freer, S. T., Xuong, Ng. H., Alden, R. A. and Kraut, J. (1973) J. Biol. Chem. 248, 3910–3921
3 Bartsch, R. G. (1976) in The Photosynthetic Bacteria (Clayton, R. K. and Sistrom, W. R., eds.), Plenum Publishing Corporation, New York
4 Ambler, R. P. (1975) in Handbook of Biochemistry and Molecular Biology (Fasman, G. D., ed.), 3rd edn., Chemical Rubber Company, Cleveland
5 Ambler, R. P., Meyer, T. E. and Kamen, M. D. (1976) Proc. Natl. Acad. Sci. U.S., in the press
6 Kamen, M. D. and Vernon, L. P. (1955) Biochim. Biophys. Acta 17, 10–22
7 Evans, E. H. and Crofts, A. R. (1974) Biochim. Biophys. Acta 357, 78–88
8 Orlando, J. A. (1967) Biochim. Biophys. Acta 143, 634–636
9 Dutton, P. L., Petty, K. M., Bonner, H. S. and Morse, S. D. (1975) Biochim. Biophys. Acta 387, 536–556
10 Morita, S. and Conti, S. F. (1963) Arch. Biochem. Biophys. 100, 302–307
11 DeKlerk, H., Bartsch, R. G. and Kamen, M. D. (1965) Biochim. Biophys. Acta 97, 275–280
12 Bartsch, R. G. (1971) in Methods in Enzymology (San Pietro, A., ed.), Vol. 23, pp. 344–363, Academic Press, New York
13 Davenport, H. E. and Hill, R. (1952) Proc. R. Soc. Lond. Ser. B 139, 327–345
14 Velick, S. F. and Strittmatter, P. (1965) J. Biol. Chem. 221, 265–275
15 Hanania, G. I. H., Irvine, D. H., Eaton, W. A. and George, P. (1967) J. Phys. Chem. 71, 2022–2030
16 Morton, R. K. (1958) Rev. Appl. Chem. 8, 161–220
17 Kolthoff, I. M. and Tomsicek, W. J. (1935) J. Phys. Chem. 39, 945–958
18 O'Reilly, J. E. (1973) Biochim. Biophys. Acta 292, 509–515

19 Clark, W. M. (1960) in Oxidation Reduction Potentials of Organic Systems, William and Wilkins, Baltimore, Md.
20 Harbury, H. A. (1957) J. Biol. Chem. 1009–1024
21 Ricard, J., Mazza, G. and Williams, R. J. P. (1972) Eur. J. Biochem. 28, 566–578
22 Kakuno, T., Hosoi, K., Higuti, T. and Horio, T. (1973) J. Biochem. Tokyo 74, 1193–1203
23 Kassner, R. J. (1972) Proc. Natl. Acad. Sci. U.S. 69, 2263–2267
24 Kassner, R. J. (1973) J. Am. Chem. Soc. 95, 2674–2677
25 Davis, L. A., Schejter, A. and Hess, G. A. (1974) J. Biol. Chem. 249, 2624–2632
26 Stellwagen, E., Babul, J. and Wilgus, H. (1975) Biochim. Biophys. Acta 405, 115–121
27 Pettigrew, G. W., Aviram, I. and Schejter, A. (1975) Biochem. Biophys. Res. Commun., in the press
28 Schechter, E. and Saludjian, P. (1967) Biopolymers 5, 788–790
29 Gupta, R. K. and Koenig, S. H. (1971) Biochem. Biophys. Res. Commun. 45, 1134–1143
30 Dayhoff, M. O. (1972) Atlas of Protein Sequence and Structure Vol. 4, pp. D11–D27, National Biomedical Research Foundation, Washington, D.C.
31 Keller, R., Aviram, I., Schejter, A. and Wüthrich, K. (1972) FEBS Lett. 20, 90–92
32 Wilgus, H. and Stellwagen, E. (1974) Proc. Natl. Acad. Sci. U.S. 71, 2892–2894
33 Margalit, R. and Schejter, A. (1973) Eur. J. Biochem. 32, 492–499
34 Pettigrew, G. W., Aviram, I. and Schejter, A. (1975) Biochem. J. 149, 155–167
35 Yamanaka, T. (1972) Adv. Biophys. 3, 227–276