Biochimica et Biophysica Acta, 292 (1973) 677–684

BBA 46496

REDISTRIBUTION OF ELECTRIC CHARGE ACCOMPANYING PHOTOSYNTHETIC ELECTRON TRANSPORT IN *CHROMATIUM*

GEORGE D. CASE* and WILLIAM W. PARSON**

Department of Biochemistry, University of Washington, Seattle, Wash. 98195 (U.S.A.)

(Received September 18th, 1972)

SUMMARY

The isoionic pH of *Chromatium* chromatophores is 5.2±0.1. At pH 7.7, the net charge on the chromatophore is approx. $-1 \cdot 10^4$. If a change in this charge accompanies the oxidation of an electron carrier, the midpoint redox potential (E_m) of that carrier should be a function of the solution ionic strength (I).

The E_m values of P870 and cytochrome *c*-555 increase strongly with increasing I at low values of I. The E_m of cytochrome *c*-552 also increases with increasing I, though not so strongly. These effects probably cannot be attributed to an influence of I on the activity coefficient of a dissociable ion. We conclude that, when either P870 or cytochrome *c*-555 loses an electron, no specific ions (including protons) are bound or released in significant amounts, and the absolute value of the charge on the chromatophore decreases.

The E_m values of the primary and secondary electron acceptors, X and Y, do not depend on I. Because these E_m values have been shown previously to depend on pH, we conclude that the uptake of a proton keeps the charge on the chromatophore constant when either X or Y accepts an electron. This means that the primary and secondary electron transfer reactions in *Chromatium* result in a net decrease in the charge on the photosynthetic membrane. They do not result in the translocation of protons across the membrane.

The E_m of the soluble flavocytochrome *c*-552 from *Chromatium* depends only weakly on I, but depends strongly on the pH. The uptake of a proton appears to keep the net charge on this cytochrome constant upon reduction.

INTRODUCTION

According to the chemiosomotic model of energy coupling, electrostatic effects may be critical to the mechanism of oxidative and photosynthetic phosphorylation[1,2]. Among the phenomena that have been attributed to a redistribution of electrical charge accompanying electron transfer are: (1) changes in the binding or transport of organic ions[3–8]; (2) shifts in the absorption spectra of endogenous pigments[9–13];

Abbreviations: E_m, midpoint redox potential; PMS, *N*-methylphenazonium methosulfate.

* Present address: Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pa. 19104, U.S.A.

** To whom correspondence should be addressed.

(3) changes in the electrophoretic mobility of chloroplasts[15]; (4) volume changes[16]; (5) structural changes[17]; and (6) changes in delayed luminescence[18,19]. Most of these observations are open to alternative interpretations.

Schejter and Margalit[20] have used the influence of ionic strength on the midpoint redox potential of mitochondrial cytochrome *c* to determine the change in electrical charge which accompanies oxidation of the cytochrome in solution. An increase in the net charge from +7.6 to +8.3 occurred upon oxidation in the presence of sodium cacodylate, but a decrease from +7.6 to +5.0 in the presence of NaCl. Schejter and Margalit[20] attributed the charge decrease in the latter case to chloride binding by the ferricytochrome. The present work adapts this approach to *Chromatium* chromatophores, in an attempt to investigate the light-driven redistribution of charges on the primary and secondary photochemical electron carriers. For comparison, we also have studied flavocytochrome *c* 552, which is a soluble, *c*-type cytochrome from *Chromatium*, with a molecular weight of 72000[21-23].

The midpoint redox potential (E_m) of an electron carrier depends on the ratio of the activity coefficients (f_o and f_r) of the oxidized and reduced forms of the carrier. For small, spherically symmetrical ions, and even for some globular proteins in solution,

$$\ln (f_o/f_r) = (z_r^2 - z_o^2) \frac{0.51\sqrt{I}}{1 + 0.33A\sqrt{I}} \quad (1)$$

where I is the ionic strength, z_o and z_r are the net electric charges on the oxidized and reduced forms of the electron carrier, and A is the ionic exclusion radius in Å[24,25]. Because an electron carrier in the chromatophore membrane may not communicate directly with the solution, and because the distribution of charges on the chromatophore is unknown, the dependence of $\ln (f_o/f_r)$ on I is undoubtedly more complex in this case. Nonetheless, the qualitative effect of ionic strength may be sufficient to reveal whether the net charge increases, decreases, or remains constant during oxidation of an electron carrier. At low values of I, the E_m of the carrier should increase with increasing ionic strength if the net charge decreases on oxidation. In addition, if binding or release of a specific ion accompanies the oxidation, varying the concentration of that ion will affect the E_m of the electron carrier strongly.

EXPERIMENTAL

Chromatium vinosum (*Chromatium* strain D) was grown photoautotrophically in the medium of Case and Parson[26]. Chromatophores were prepared by sonication of the cells in 0.1 M potassium phosphate buffer containing 0.3 M sucrose, followed by differential centrifugation. Flavocytochrome *c*-552 was prepared according to the procedure of Bartsch and Kamen[22]. Prior to use, the flavocytochrome *c*-552 was dialyzed exhaustively against distilled water.

Measurements of photooxidation of P870 and cytochrome after each of two saturating laser flashes were used in the redox titrations of P870, cytochrome *c* 555, and the primary and secondary electron acceptors, X and Y[26]. The titrations of cytochrome *c*-552 and flavocytochrome *c*-552 employed steady-state measurements, without actinic illumination[26]. The redox potential was measured with a Pt electrode and a calomel reference electrode, as described previously[26]. For all of the redox titrations of chromatophores, the bacteriochlorophyll concentration was approx.

44 μM. The temperature was 22 °C, except as noted in the figure legends. The ionic strength was varied by changing the concentration of potassium phosphate or (as indicated in the figure legends) Tris–HCl, at pH 7.70. Sucrose was present (0.3 M) when the ionic strength was less than 0.2; sucrose had no effect on the E_m values at higher ionic strengths. Redox buffers used were 50 μM $K_3Fe(CN)_6$ (for P870), 100 μM *N*-methylphenazonium methosulfate (PMS) (for cytochrome *c*-555), 50 μM PMS *plus* 50 μM methylene blue (for cytochrome *c*-552 and flavocytochrome *c*-552), and 50 μM indigo disulfonate (for X and Y).

Before the redox titrations were begun, the chromatophore suspensions were allowed to equilibrate in the dark at room temperature for at least 1 h. We assumed that the ionic strength was essentially the same on both sides of the chromatophore membrane after this equilibration.

Before reductive titrations of P870, the chromatophore suspensions were oxidized by titration with 5 mM K_4IrCl_6, until no P870 photooxidation could be detected. A similar oxidation with $K_3Fe(CN)_6$ preceeded the cytochrome *c*-555 titrations. Reductive titrations then were carried out with 2 mM $Na_2S_2O_4$ in 5 mM NaOH, or (when the ionic strength was below 0.03) with 5 mM dithiothreitol. Back titrations also were performed, with air as the oxidant. The contributions of the K_4IrCl_6, $K_3Fe(CN)_6$, and the reductant were included in the calculations of I.

For measurements of the net charge as a function of pH, samples were deionized by successive passages over 0.5 cm × 9 cm columns of Amberlite MB-1 (Rohm and Haas Co., Philadelphia), until further passages ceased to alter the pH of the effluent. One such column was sufficient to deionize a 0.8 μM flavocytochrome *c*-552 solution. Suspensions of chromatophores, containing approx. 2 mM bacteriochlorophyll in 0.4 M deionized sucrose, required passage over 4–6 columns. Following this treatment, resonication of the chromatophore suspension or the addition of gramicidin D did not alter the pH of the effluent suspensions from subsequent columns. The pH of the final effluent was taken as the isoionic pH, and samples were titrated from that point to pH 10 with standard NaOH, and back with standard HCl. Artifacts due to contamination from atmospheric CO_2 were measured by titrating 0.4 M sucrose which had been deionized in the same manner. The concentration of photosynthetic reaction centers in the chromatophore suspension was estimated from the amount of cytochrome *c*-555 photooxidation which resulted from a single, saturating actinic flash, by assuming $\Delta\varepsilon^{red-ox}_{422\,nm} = 103\ mM^{-1}\cdot cm^{-1}$ (refs 27 and 28). Each chromatophore contains about 12 photosynthetic reaction centers[19].

RESULTS

Flavocytochrome c-552

By titration of solutions of flavocytochrome *c*-552 from the isoionic point (pH 4.9), we measured a value of $z_o = -13 \pm 1$ at pH 7.70. If reduction of one of the two hemes on the cytochrome involved a change of -1 in the electric charge, the E_m should increase with increasing ionic strength. Although the observed effect is in this direction (Fig. 1, right), the dependence of the E_m on ionic strength is too low to be consistent with a change of -1 in the charge. If $z_o = -13$, the observed dependence corresponds to a change in the electric charge of only -0.24 upon

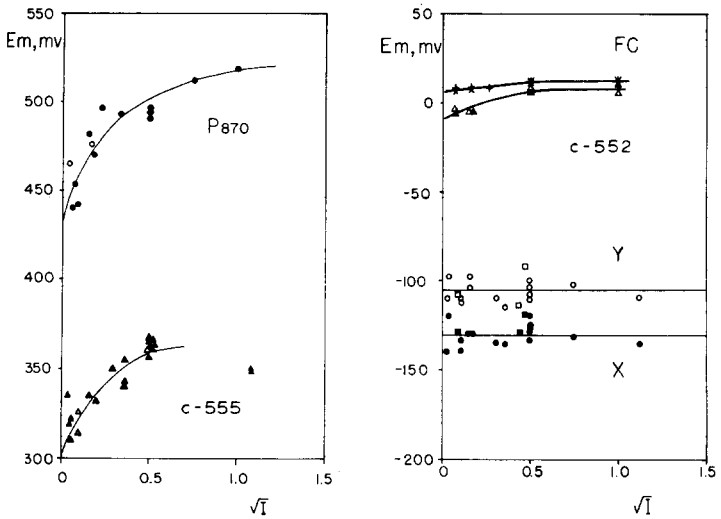

Fig. 1. Left: Ionic strength dependence of the redox titrations of P870 and cytochrome *c*-555. *I* values do not include the contribution from the chromatophores. ●, P870 E_m values; *I* varied with potassium phosphate. ○, same, except with Tris–HCl. ▲, cytochrome *c*-555 midpoint potentials as measured with single actinic flashes; the true E_m values are about 20 mV more negative than these potentials[26]. *I* varied with potassium phosphate. △, same, except with Tris–HCl. Right: Ionic strength dependence of the redox titrations of cytochrome *c*-552, *X*, *Y*, and flavocytochrome *c*-552 (FC). *I* values do not include the contribution from the chromatophores; that from flavocytochrome *c*-552 was negligible. ●, E_m values for *X*; *I* varied with potassium phosphate. ■, same, except with Tris–HCl. ○, midpoint potentials for *Y*, as measured by cytochrome oxidation on a second actinic flash; the true E_m values are about 10 mV more positive than these potentials[26]. *I* varied with potassium phosphate. □, same, except with Tris–HCl. ▲, cytochrome *c*-552 E_m values; *I* varied with potassium phosphate. △, same except 22 °C intercepts of E_m values measured at different temperatures between 0 and 40 °C. +, Flavocytochrome *c*-552 E_m values; *I* varied with potassium phosphate. ×, same, except 22 °C intercepts of E_m values measured at different temperatures between 0 and 40 °C.

reduction of the cytochrome. (For this calculation, we used Eqn 1, assuming $A = 33$Å, typical of a globular protein of this size[25]). Apparently, uptake or release of an ion accompanies the reduction of flavocytochrome *c*-552.

The insensitivity of the E_m to variation in the concentration of K^+ or phosphate (Fig. 1) also indicates that no change in the binding of either of these ions accompanies the reduction of flavocytochrome *c*-552. Except for ions that may have been introduced to the solution already bound to the cytochrome, H^+ and OH^- were the only other ions present in significant concentration in these experiments. Fig. 2 shows that the E_m of the flavocytochrome decreases with increasing pH, by 43 mV per pH unit. The slope corresponds to the uptake of approximately 0.75 H^+ per electron. We conclude that the binding of a proton keeps the electric charge of flavocytochrome *c*-552 essentially constant during reduction.

Because the soluble flavocytochrome *c*-552 and the membrane-bound cytochrome *c*-552 have similar spectra and E_m values, several investigators[23,26,30,31] have suggested that the two cytochromes might be identical. Our present results indicate that the similarity in E_m values for the two cytochromes is coincidental.

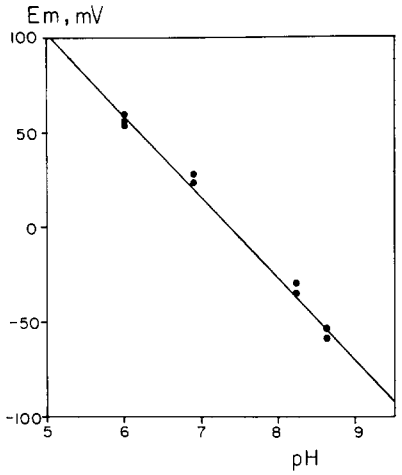

Fig. 2. pH dependence of the flavocytochrome *c*-552 E_m values. The buffer was 0.1 M potassium phosphate or, for pH values above 8.0 or below 6.5, 0.1 M Tris–citrate. The line was fit to the points by linear regression.

While the E_m for flavocytochrome *c*-552 varies significantly with pH, E_m values for the chromatophore-bound cytochrome *c*-552 are independent of pH[26]. This observation extends other recent reports[27,32,33] of differences between the two cytochromes.

P870 and cytochrome c-555

Chromatium chromatophores are isoionic at pH 5.2 ± 0.1. At pH 7.7, the net charge on the chromatophore is -800 ± 60 per reaction center, or approximately $-1 \cdot 10^4$ per chromatophore. The $\pm$ 8% uncertainty in net charge results mainly from hysteresis in the forward and backward titrations with NaOH and HCl. This effect may be due to slowness of diffusion of H^+ through the chromatophore membrane.

Fig. 1 (left) shows the effect of ionic strength on the midpoint redox potentials of cytochrome *c*-555 and P870 in *Chromatium* chromatophores. At low ionic strength, the E_m values for both electron carriers increase markedly with increasing ionic strength. The data in Fig. 1 do not take account of the contribution that the chromatophores themselves made to the ionic strength. If one includes this contribution of approx. 0.06 M, the E_m values at low ionic strength actually are a much stronger function of $\sqrt{I}$ than the figure suggests.

Titrations in which either Tris–HCl or potassium phosphate served as the principal salt gave similar results (Fig. 1). Furthermore, varying the concentration of chromatophores by a factor of 20 has no effect on cytochrome *c*-555 titrations at a solution ionic strength of 0.25, indicating that the E_m of the cytochrome does not depend on dissociable ions which are introduced to the solution with the chromatophores. These observations argue against the possibility that the sensitivity of the cytochrome *c*-555 titrations to ionic strength reflects the activity coefficient of a dissociable ion. The most direct interpretation of the results is that oxidation of either P870 or cytochrome *c*-555 results in a decrease in the negative charge on the

chromatophore, simply due to the loss of an electron. The insensitivity of the cytochrome *c*-555 titrations to pH[26] supports this view.

One observation that this conclusion does not explain is the apparent lack of electrostatic interaction between the two cytochrome *c*-555 hemes[26]. A second difficulty is that titrations of P870 do show some dependence on pH, though less than that of the X and Y titrations[26].

Cytochrome c-552

The right half of Fig. 1 shows that the E_m of cytochrome *c*-552 in chromatophores increases slightly with increasing ionic strength. The ionic strength dependence of the cytochrome *c*-552 titrations is much less striking than that for the cytochrome *c*-555 titrations, but greater than that for the redox titrations of the soluble flavocytochrome *c*-552. Because the E_m of cytochrome *c*-552 does not depend on pH[26], the net charge on the chromatophore also should decrease as this cytochrome loses an electron. This might not occur if some other dissociable ion participates in the reaction, but the data rule out the participation of K^+ or phosphate ions, and no other ions were added in significant amounts.

X and Y

As the right half of Fig. 1 indicates, the midpoint redox potentials of the primary and secondary electron acceptors, X and Y, are altogether independent of ionic strength. This finding supports the conclusion that the uptake of a proton[26,34,35] keeps the net electric charge on the chromatophore constant during reduction of either X or Y. The observation[26] that the redox state of X appears to have no effect on the cytochrome *c*-555 titrations is consistent with this interpretation.

DISCUSSION

The light-driven transfer of an electron from cytochrome *c*-555 to X or Y would appear to leave the photosynthetic reaction center with one less negative charge than it possesses in the ground state. The uptake of a proton evidently compensates for the appearance of an electron on X or Y, and there is no indication that any other ions are bound or released. The electrostatic changes that accompany P870 oxidation appear to be similar to those that accompany cytochrome *c*-555 oxidation.

Several investigators[9,10,12,18,19] have proposed that the primary electron donor and acceptor occupy opposite surfaces of the photosynthetic membrane. In their view, the primary photochemical reaction generates a transverse dipole in the membrane. Proton binding and release by electron carriers on opposite sides of the membrane subsequently creates a uniform transmembrane electrical potential, or "membrane potential". In the chemiosmotic theory[1,2], it is a membrane potential that drives the energy-linked functions of mitochondria, chloroplasts, and chromatophores.

Contrary to this model, the present study indicates that the primary and secondary photochemical reactions do not result in a translocation of protons across the membrane. Even if the H^+ which binds to X^- or Y^- comes exclusively from one side of the membrane, P870 and the cytochromes evidently do not release a

proton, or any other specific ion, to the solution on the other side. Nonetheless, capacitive coupling between the solution and P870 or the cytochromes may still allow the photochemical reactions to generate a membrane potential. Changes in the electrical charge on the electron carriers must cause a realignment of ions in the solution nearby, for it is this realignment that underlies the effect of the ionic strength on the E_m values. Because charges on P870 and the cytochromes also must couple capacitively to the solution on the farther side of the membrane, and to internal components of the membrane, one cannot predict the magnitude of the resulting membrane potential. One can say only that the membrane potential must be smaller than the potential that would result from the net translocation of an ion from one solution to the other. It is possible, of course, that ion translocation accompanies the electron transfer reactions that follow the primary and secondary reactions. The investigation of these slower steps remains a major problem of bacterial photosynthesis.

ACKNOWLEDGEMENTS

We are indebted to Dr Thomas Horbett for advice on the acid–base titrations. National Science Foundation Grants GB 13495 and GB 30732X supported this research.

REFERENCES

1 Mitchell, P. (1966) *Chemiosomotic Coupling in Oxidative and Photosynthetic Phosphorylation*, Glynn Research Ltd, Bodmin, Cornwall
2 Mitchell, P. and Moyle J. (1969) *Eur. J. Biochem.* 7, 471–484
3 Azzi, A. (1969) *Biochem. Biophys. Res. Commun.* 37, 254–260
4 Azzi, A., Baltscheffeky, M., Baltscheffsky, H. and Vainio, H. (1971) *FEBS Lett.* 17, 49–52
5 Dell'Antone, P., Colonna, R. and Azzone, G. F. (1971) *Biochim. Biophys. Acta* 234, 541–544
6 Jasaitis, A. A., Kuliene, V. V. and Skulachev, V. P. (1971) *Biochim. Biophys. Acta* 234, 177–181
7 Kraayenhof, R. (1970) *FEBS Lett.* 6, 161–165
8 Liberman, E. A. and Skulachev, V. P. (1970) *Biochim. Biophys. Acta* 216, 30–42
9 Jackson, J. B. and Crofts, A. R. (1969) *FEBS Lett.* 4, 185–189
10 Jackson, J. B. and Crofts, A. R. (1971) *Eur. J. Biochem.* 18, 120–130
11 Junge, W. (1970) *Eur. J. Biochem.* 14, 582–592
12 Junge, W. and Witt, H. T. (1968) *Z. Naturforsch.* 23b, 244–254
13 Junge, W., Rumberg, B. and Schroder, H. (1970) *Eur. J. Biochem.* 14, 575–581
14 Strichartz, G. R. and Chance, B. (1972) *Biochim. Biophys. Acta* 256, 71–84
15 Nobel, P. S. and Mel, H. C. (1966) *Arch. Biochem. Biophys.* 113, 695–702
16 Callis, J. B., Parson, W. W. and Gouterman, M. P. (1972) *Biochim. Biophys. Acta* 267, 348–362
17 Green, D. E. and Ji, S. (1972) *Proc. Natl. Acad. Sci. U.S.* 69, 726–729
18 Crofts, A. R., Wraight, C. A. and Fleischman, D. E. (1971) *FEBS Lett.* 15, 89–100
19 Fleischman, D. E. (1971) *Photochem. Photobiol.* 14, 277–286
20 Schejter, A. and Margalit, R. (1970) *FEBS Lett.* 10, 179–181
21 Bartsch, R. G., Coval, M. L. and Kamen, M. D. (1961) *Biochim. Biophys. Acta* 51, 241–245
22 Bartsch, R. G. and Kamen, M. D. (1960) *J. Biol. Chem.* 235, 825–831
23 Bartsch, R. G., Meyer, T. E. and Robinson, A. B. (1968) in *Structure and Function of Cytochromes* (Okunuki, K., Kamen, M. D. and Sezuku, I., eds), pp. 443–451, University of Tokyo Press, Tokyo
24 Fowler, R. H. and Guggenheim (1939) *Statistical Thermodynamics* pp. 377–420, Cambridge University Press, Cambridge
25 Tanford, C. (1960) *Physical Chemistry of Macromolecules*, pp. 457–525, Wiley, New York

26 Case, G. D. and Parson, W. W. (1971) *Biochim. Biophys. Acta* 253, 187–202
27 Kennel, S. J. and Kamen, M. D. (1971) *Biochim. Biophys. Acta* 253, 153–166
28 Parson, W. W. (1968) *Biochim. Biophys. Acta* 153, 248–259
29 Cusanovich, M. A. and Kamen, M. D. (1968) *Biochim. Biophys. Acta* 153, 376–396
30 Cusanovich, M. A., Bartsch, R. G. and Kamen, M. D. (1968) *Biochim. Biophys. Acta* 153, 397–417
31 Seibert, M. and DeVault, D. (1970) *Biochim. Biophys. Acta* 205, 220–231
32 Kennel, S. J. and Kamen, M. D. (1971) *Biochim. Biophys. Acta* 234, 458–467
33 Hendricks, R. and Cronin, J. R. (1971) *Biochem. Biophys. Res. Commun.* 44, 313–318
34 Chance, B., Crofts, A. R., Nishimura, M. and Price, B. (1970) *Eur. J. Biochem.* 13, 364–374
35 Cogdell, R. J., Jackson, J. B. and Crofts, A. R. (1973) *J. Bioenerg.*, in the press.