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Electrogenic events in chromatophores from *Rhodobacter sphaeroides* lacking high-potential cytochrome *b* of the bc_1-complex

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The ubiquinol:cytochrome *c* oxidoreductase (bc_1-complex), contains a cytochrome (cyt) *b* subunit, which contributes to both quinol oxidizing (Q_Z) and quinone reductase (Q_C) sites. As shown by electrochromic measurements [1,2], the antimycin-insensitive electrogenic reaction linked to electron transfer between cyt b_l and b_h contributes about 50% of the total electrogenesis of the bc_1-complex. The nature of the remaining antimycin-sensitive electrogenic step is controversial. The antimycin sensitivity of both the forward and the reverse (in the presence of myxothiazol) reaction shows that the phase is linked to reduction of Q at the Q_C site by cyt b_h [1–5]. Direct electrometry [3] suggests that the electron transfer between b_h and Q_C is not electrogenic. Probably, the antimycin-sensitive electrogenesis is at least partially due to the protonation that accompanies the reduction of Q_C. Another possible electrogenic step could be the release of protons, trapped at the Q_Z site on oxidation of QH_2, as b_h becomes oxidized [6].

The goal of the present work is to clarify these points by measurement of the flash-induced electrical potential difference ($\Delta\psi$) generated by chromatophores from *Rhodobacter sphaeroides* mutants which have lost the heme of cyt b_h. Replacement of the axial ligands of cyt b_h (His-111 by either Asp (H111D) or Asn (H111N), or His-212 by Asp (H212D)) resulted in selective loss of cyt b_h but retention of cyt b_l [7]. Flash kinetic studies of the H111N mutant showed that the myxothiazol-sensitive reactions at the Q_Z-site, including reduction of cyt b_l, were still functional.

Under oxidizing conditions ($E_h > 300$ mV) ubiquinol formation in reaction center (RC) takes place only after even-numbered flashes. Fig. 1A shows the photoelectric responses of *Rb. sphaeroides* mutant (H111N) chromatophores adsorbed on the surface of a phospholipid and ubiquinone-impregnated collodion film. After the first flash $\Delta\psi$ increases rapidly ($t < 100$ ns) as a result of primary charge separation in the RC. The second electrogenic phase (t approx. 100 μs) is due to the reduction of oxidized primary donor P by cyt c_2. Following the second flash, two additional small electrogenic phases appeared. The difference between the second and first flashes (Fig. 1B, upper curve) shows a fast phase ($t = 90$ μs), which is due to the protonation of doubly reduced Q_B in the RC [3], and a slower phase (t approx. 10 ms). Myxothiazol (Fig. 1B, lower curve) has hardly any effect on the faster of these two phases, but completely inhibits the slower phase, and reveals a small additional electrogenic phase of opposite polarity. Antimycin did not influence the photoelectric responses when added either before or after myxothiazol, but stigmatellin partially inhibited the negative phase (not shown).

Partial reactions which may contribute to the electrogenic events in the bc_1-complex are: (1) electron transfer between b_l and b_h; (2) proton uptake coupled to the redox reactions at the Q_C site; (3) electron transfer between Q_Z and b_l; (4) proton release on oxidation of QH_2 at the Q_Z site; (5) electron transfer from {2Fe/2S:cyt c_l} to cyt c_2 and (6) protolytic reactions on binding or release of QH_2 at the Q_c site. Since the chromatophores investigated lacked cy-

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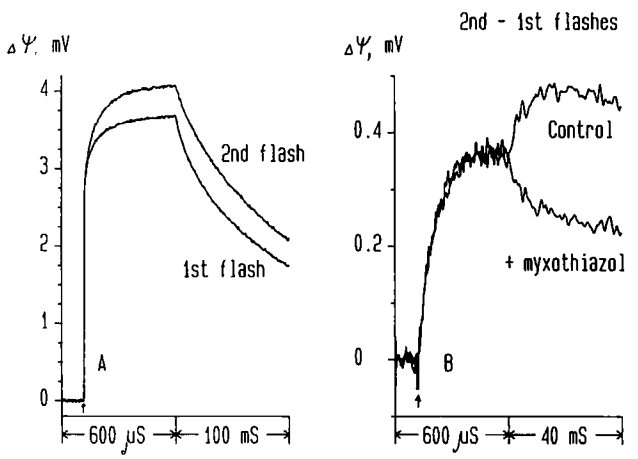

Fig. 1. Flash-induced photoelectric responses of *Rb. sphaeroides* (mutant H111N) chromatophores. Incubation medium: 30 mM Hepes (pH 7.5), 20 mM TMPD, 200 μM potassium ferrocyanide, $E_h = 300$ mV. Addition: 5 μM myxothiazol. Time between flashes was 2 s.

tochrome b_h, we can exclude reactions (1) and (2) from consideration. Reactions (3) and (4) are tightly coupled and it would be difficult to distinguish their separate contributions, but both would be inhibited by myxothiazol, and either or both could account for the myxothiazol-sensitive positive phase. Myxothiazol would not be expected to inhibit reaction (5), nor to prevent QH_2 binding at the Q_C site (reaction 6), and both reactions could contribute a myxothiazol-insensitive negative electrogenic phase. The partial inhibition by stigmatellin is consistent with a contribution from reaction (5). The lack of cyt b_h does not preclude a contribution from reaction (6), but the failure of antimycin to inhibit would require ad hoc explanations. It should be noted, that in the mutant strain, the amplitude of $\Delta\psi$ decay in the presence of myxothiazol (Fig. 1B, lower curve) was less than one half of the antimycin-sensitive negative electrogenic phase observed in wild type chromatophores [5], which has been ascribed to reversal of the reactions at the Q_C site.

The results suggest that the redox reactions at the Q_Z site of the bc_1-complex are electrogenic, but that orientation of the reactions allows partial compensation of a positive process by a negative one. The net amplitude of the electrogenic phase corresponds to a charge transfer across about 5 Å (assuming a P-Q_A distance of 27 Å [8]).

References

1 Glaser, E.G. and Crofts, A.R. (1984) Biochim. Biophys. Acta 766, 322–333.
2 Robertson, D.E. and Dutton, P.L. (1988) Biochim. Biophys. Acta 935, 273–291.
3 Drachev, L.A., Kaurov, B.S., Mamedov, M.D., Mulkidjanian, A.Ya., Semenov, A.Yu., Shinkarev, V.P., Skulachev, V.P. and Verkhovsky, M.I. (1989) Biochim. Biophys. Acta 973, 189–197.
4 Crofts, A.R., Glaser, E. and Meinhardt, S.W. (1985) in Proton Pumping in Photosynthesis and Respiration, Table Ronde Rousel-UCLAF No. 52. (Crofts, A.R., Joliot, P. and Mitchell, P., eds.), pp. 24.
5 Mulkidjanian, A.Ya., Mamedov, M.D., Semenov, A.Yu., Shinkarev, V.P., Verkhovsky, M.I. and Drachev, L.A. (1990) FEBS Lett. 277, 127–130.
6 Konstantinov, A.A. (1990) Biochim. Biophys. Acta 1018, 138–141.
7 Yun, C.-H., Crofts, A.R. and Gennis, R.B. (1991) Biochemistry 30, 6747–6754.
8 Allen, J.P., Feher, G., Yeates, T.O., Komiya, H. and Rees, D.C. (1987) Proc. Natl. Acad. Sci. USA 84, 5730–5734.