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Biochimica et Biophysica Acta 1229 (1995) 73–80

BBA Biochimica et Biophysica Acta

The electron transport system of the facultative phototroph *Rhodoferax fermentans*. I. A functional, thermodynamic and spectroscopic study of the respiratory chain of dark- and light-grown cells

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Received 7 June 1994; revised 18 November 1994; accepted 7 December 1994

Abstract

Membranes isolated from light- and dark-grown cells of the recently established new taxon of the purple nonsulfur bacteria, *Rhodoferax fermentans*, gen. nov., sp. nov., have been examined. The results have been interpreted to show that the oxidative electron transport chain is branched at the ubiquinone level and does not involve rhodoquinone. Dark-grown membranes contain four *b*-type and three *c*-type membrane-bound cytochromes with $E_{m7.0}$ of +371, +315, +76 and −18 mV and +298, +201 and +44 mV, respectively. No significant amount of soluble *c* was found in aerobic cells. Conversely, photosynthetically grown cells contain a soluble *c*-type haem (α_{max} at 551 nm, $E_{m7.0}$ = +287 mV), four membrane-bound *c*-type haems with $E_{m7.0}$ of +358, +296, +78 and −1 mV and three cytochromes *b* with $E_{m7.0}$ of +320, +30 and −50 mV. Notably, the absence of cyt *b*-371 from light-grown membranes parallels the very low rate of cyt *c* oxidase activity catalyzed by this type of membrane. Oxido-reduction kinetics demonstrated that most of the *c*-type haems detected in light-grown membranes are not involved in respiration. These data suggest that the facultative phototroph *Rf. fermentans* is endowed with an electron transport system different from that of typical facultative phototrophs, e.g., *Rhodobacter* and *Rhodospirillum* species, but similar to that of green- and purple-nonsulfur genera such as *Chloroflexus, Rhodocyclus* and *Rhodopseudomonas*.

Keywords: Facultative phototroph; Cytochrome *b*; Cytochrome *c*; Respiratory chain; Membrane-bound oxidase; (*Rf. fermentans*)

1. Introduction

Hiraishi and Kitamura [1] reported the isolation from ditchwater and activated sludge of some strains of phototrophic purple nonsulfur bacteria that were phenotypically similar to *Rhodocyclus gelatinosus*. Subsequent studies have demonstrated that these isolates differed from the latter genera in containing rhodoquinones (RQ) but not menaquinones (MK) along with their peculiarity to show a bicarbonate-stimulated fermentative growth with fumarate as a terminal oxidant [2–5]; thus they were tentatively referred to as the '*Rhodocyclus gelatinosus*-like (RGL)' group. Recently, a more complete taxonomic analysis permitted the establishment of the RGL group as a new taxon of the purple nonsulfur bacteria, for which the name *Rhodoferax fermentans* gen. nov., sp. nov. was proposed [6]. In contrast to other facultative phototrophs such as *Rhodobacter* and *Rhodopseudomonas* species, the synthesis of bacteriochlorophyll (BChl) *a* and carotenoids of the spheroidene series in *Rf. fermentans* is not coupled to the formation of an apparent intracytoplasmic membrane system (ICM) [6,7]. Conversely, in a similar way to *Rhodospirillum* species, both ubiquinone-8 (UQ-8) and rhodoquinone-8 (RQ-8) occur as major quinones under variable cultural conditions; however, while in cells grown anaerobically in the dark or in the light the RQ/UQ molar ratio is between 0.9 and 1.7, the ratio is much lower (0.1–0.2) in aerobically grown cells [6]. These features suggest that *Rf. fermentans*, although sharing some of the

Abbreviations: DAD, 3,6-diaminodurene; DCIP, 2,6-dichloroindophenol; Menaquinone (MQ), 2-polyprenylmethyl-1,4-naphthoquinone; Rhodoquinone (RQ), 2-methoxy-3-amino-5-methylpolyprenyl-1,4-benzoquinone.

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SSDI 0005-2728(94)00189-8

Table 1
Oxidative activities [a] of membranes from aerobically grown cells of *Rf. fermentans*

Electron donors	Electron acceptors	Activities (% of control activity)					
		–	Myxo	AA	Stig	Rot	Pier
1. NADH	O_2	1.56 (100)	0.65 (42)	1.56 (100)	0.89 (57)	1.50 (96)	0.30 (20)
2. NADH	DCIP	19.3 (100)	–	–	–	17.4 (90)	17.4 (90)
3. NADH	cyt *c*	2.85 (100)	1.48 (52)	2.10 (74)	1.96 (69)	–	–
4. Succinate	O_2	0.21 (100)	0.10 (50)	0.18 (86)	0.11 (55)	–	–
5. Succinate	DCIP	1.16 (100)	–	–	–	–	–
6. Succinate	cyt *c*	0.91 (100)	0.45 (50)	0.68 (75)	0.02 (2)	–	–
7. Asc-DAD	O_2	6.90 (100)	–	–	–	–	–

[a] Expressed as μmol of acceptor reduced h^{-1} (mg protein)$^{-1}$.
Additions and non-standard abbreviations: NADH (0.2 mM); succinate (2 mM); ascorbate-DAD (Asc/DAD, 5 mM/0.25 mM); DCIP (0.25 mM); cyt *c* (0.05 mM); myxothiazol (Myxo, 5 μM); antimycin (AA, 5 μM); rotenone (Rot, 5 μM); stigmatellin (Stig, 5 μM); piericidin (Pier, 5 μM).

characteristics of the genera *Rhodobacter, Rhodopseudomonas* and *Rhodospirillum*, might be of special interest from a biochemical and physiological point of view.

Here we report the first spectroscopic and thermodynamic study of membranes isolated from cells of *Rf. fermentans* grown in the dark (aerobically) and in the light (anaerobically). In addition, oxidative activities catalysed by these two types of membrane have been examined. The results have been interpreted to show that the plasma-membrane of *Rf. fermentans* contain a branched respiratory chain composed only of cytochromes of *b*- and *c*-type. Significantly, although several *c*-type haems were shown to be involved in oxidative electron transport, significant amounts of a soluble cytochrome *c* (α band maximum at 551 nm, $E_{m7.0} = +287$ mV) were found in phototrophically grown cells only. This latter finding indicates similarities between the electron transport chain of light-grown *Rps. viridis* and that of *Rf. fermentans* [8].

Preliminary results of the present work have recently been presented during the Workshop on Structure-Function of Ion-Translocating Complexes (Oct 3–7, 1993, Freiburg, Germany).

2. Materials and methods

2.1. Organism and cultivation

Aerobic growth of *Rhodoferax fermentans* JMC 7819 (a generous gift of Dr. A. Hiraishi, Konishi Co. Ltd., Tokyo) was carried out in the dark at 30° C in MYCA medium as described previously [1]. Anaerobic liquid cultures were made in completely filled screw-capped test-tubes or bottles with an incident light intensity of 0.4 W cm^{-2}.

2.2. Membrane isolation

Cells were harvested at late logarithmic growth phase (0.6–0.7 absorbance at 660 nm). After washing with 3-[*N*-morpholino]propanesulfonic acid, 5 mM $MgCl_2$ buffer (pH 7.2), membrane fragments were prepared by following the French pressure-cell method in the presence of DNase I (Fluka) ($15 \cdot 10^3$ units (g cells wet wt.)$^{-1}$) [9]. Supernatants were obtained and analyzed as in Prince and Daldal [10].

Table 2
Oxidative activities of membranes from phototrophically grown cells of *Rf. fermentans*

Electron donor	Electron acceptors	Activity (% of control)					
		–	Myxo	AA	Stig	Rot	Pier
1. NADH	O_2	0.47 (100)	0.31 (67)	0.47 (100)	0.28 (60)	0.42 (90)	0.23 (50)
2. NADH	DCIP	27.0 (100)	–	–	–	21.6 (80)	27.0 (100)
3. NADH	cyt *c*	3.2 (100)	2.65 (83)	2.91 (91)	2.65 (83)	–	–
4. Succinate	O_2	0.1 (100)	0.07 (68)	0.09 (88)	0.07 (70)	–	–
5. Succinate	DCIP	1.00 (100)	–	–	–	–	–
6. Succinate	cyt *c*	1.27 (100)	0.61 (48)	1.01 (80)	0.12 (10)	–	–
7. Asc-DAD	O_2	3.66 (100)	–	–	–	–	–
8. Asc-DAD [a]	O_2	2.02 (100)	2.42 (120)	–	–	–	–

Experimental conditions and additions as for Table 1. Activitites as μmol O_2 reduced h^{-1} (mg protein)$^{-1}$.
[a] This activity was measured under aerobic, illuminated, conditions (0.8 W cm^{-2}) after subtraction of the ascorbate-DAD oxidation rate resistant to CN^- 10 μM and is referred to as light-induced oxygen uptake (see Zannoni et al. [9]) or light-activated quinol oxidase (this work). It quantifies the amount of O_2 consumed when reducing equivalents from exogenously added donors are delivered to oxygen through reversal of the electron flow by the photochemical reaction centre (RC), i.e., ascorbate-DAD → RC (light) → QH^2 → oxygen.

2.3. Analytical procedures

Optical spectroscopy, polarography, redox potentiometry and oxido-reduction kinetics followed standard methods [11]. Estimation of the amounts of cytochromes was made by using the following extinction coefficients and wavelength pairs: cyt *c*, 550–540 nm, $\epsilon = 18.6$ mM^{-1} cm^{-1}; cyt *b*, 560–575 nm, $\epsilon = 21$ mM^{-1} cm^{-1}. Light-induced oxygen uptake was measured as described previously [9]. Protein concentration was determined by the Bradford method [12] and bacteriochlorophyll was measured after extraction with acetone/methanol (7:2, v/v) using an ϵ of 75 mM cm^{-1} at 775 nm [13].

3. Results

3.1. Oxidation of β-NADH, succinate and reduced DAD

Tables 1 and 2 show that membranes isolated from dark- and light-grown cells of *Rf. fermentans* catalyze several respiratory activities. Oxidation of β-NADH in both types of membrane was completely inhibited by 10 mM CN^- (not shown) but insensitive to 5 μM rotenone (row 1). Further experiments indicated that aerobic membranes readily oxidized deamino-NADH, this analogue being indicative of an NDH-1 type enzyme [14]. Accordingly, oxidation of β-NADH was inhibited by 5 μM piericidin (80 and 50% inhibition in dark- and light-grown membranes, respectively). Conversely, the NADH-DCIP oxidoreductase activity, which is operationally taken as indicative of the NADH dehydrogenase activity, was largely insensitive to rotenone and piericidin in both dark- and light- grown cells (row 2, Tables 1 and 2). Respiration was also insensitive to antimycin A but inhibited, although not completely, by myxothiazol and/or stigmatellin (5 μM each). The data of Tables 1 and 2 also show that the cytochrome *c* oxidase (measured as ascorbate-DAD oxidation) of light-grown cells was approx 2.5-times lower than the analogous activity in dark-grown membranes.

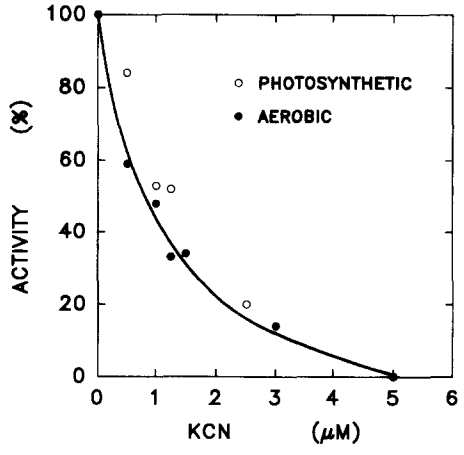

Fig. 1. Cyanide inhibition of the ascorbate-DAD oxidation activities in membranes from dark- and light-grown cells of *Rf. fermentans*. See text for further details.

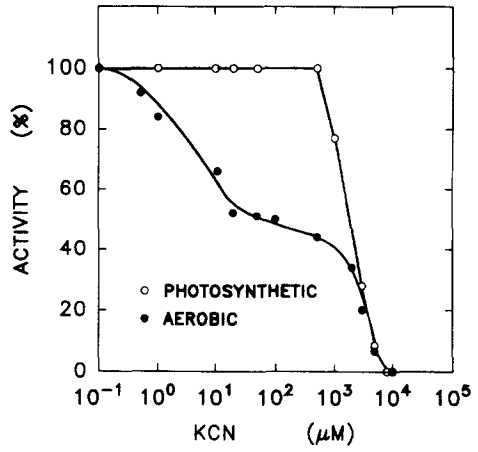

Fig. 2. Cyanide inhibition of the succinate oxidase activities in membranes from dark- and light-grown cells. See text for details.

It is well established that several facultative phototrophs are endowed with a branched respiratory chain [15]. To test the presence of a branched electron transport system in membranes from *Rf. fermentans*, the respiratory activities coupled to oxidation of ascorbate/DAD and/or succinate were measured as a function of variable CN^- concentrations. Fig. 1 shows that 5 μM CN^- fully inhibits the cyt *c* oxidase activities in both dark- and light-grown membranes ($I_{50\%} = 10^{-6}$ M). Interestingly, the succinate-dependent respiration was 50% inhibited by 5 μM CN^- only in aerobic membranes whereas light-grown membranes were unaffected by this CN^- concentration (shown in Fig. 2, filled and open circles, respectively). This result suggests that in anaerobically light-grown cells the oxidase which is sensitive to low CN^- concentrations is not rate-limiting when reducing equivalents originate from the different dehydrogenases. In both light- and dark-grown membranes, however, respiration was completely blocked by 10^{-2} M CN^-. Apparently, the succinate-dependent oxidase activity blocked by 10^{-5} M CN^- corresponds to the ascorbate/DAD oxidase of Fig. 1, whereas the oxidase activity inhibited by 10^{-2} M CN^- is likely to be catalyzed by a quinol oxidase similar to those observed in other purple nonsulfur bacteria [15] (see also below). The $I_{50\%KCN}$ of the two oxidases seen in dark-grown cells were 2.5 μM and 2.5 mM, respectively.

3.2. Quinol oxidation

Previous studies have demonstrated that, in membranes from several species of facultative phototrophs, a good rate of oxygen consumption can be driven by light when reducing equivalents from exogenously added donors are delivered to O_2 through reversal of the electron flow by the photochemical reaction centre (RC), i.e., reduced-DAD

→ RC → QH_2 → oxidase [9,16]. This light – driven respiration, which is resistant to CN^- concentrations inhibiting the cyt *c* oxidase activity, is referred to as "light-induced oxygen uptake" or "light-activated quinol oxidase". Experiments 8 and 1 of Tables 2 and 3, respectively, show that *Rf. fermentans* photosynthetic membranes can reduce oxygen in the light (0.8 W cm^{-2}) under experimental conditions in which the cyt *c* oxidase is blocked by 10 μM CN^-. This result, along with those of Tables 1 and 2, indicates that *Rf. fermentans* is endowed with a branched electron transport chain in which reducing equivalents from ascorbate-DAD (in the light), or succinate and NADH (in the dark), are delivered to a quinol oxidase containing pathway. To test the possible role of rhodoquinone (E'_o = −30 mV, Ref. [30]) in catalyzing the quinol oxidase activity of *Rf. fermentans* light-grown membranes (RQ/UQ ratio between 0.9 and 1.7), the light-induced oxygen uptake was measured in the presence of exogenously added quinones having different redox potentials (Table 3). Apparently, the photoactivated quinol oxidase activity was drastically stimulated (223%) by the short-chain quinone Q_2 (E_m = +112 mV) and partially inhibited (37%) by Q_0 (E_m = +162 mV). No significant stimulation was observed with 1,4-naphthoquinone (E_m = +65 mV), duroquinone (E_m = +35 mV) and the most electronegative quinone, menadione (E_m = −1 mV). These results were supported by further experiments in which photosynthetic membranes were tested for respiration with the quinols listed in Table 3 as electron donors. The quinols were generated from the corresponding quinones by reduction with NADH in the presence of D,T-diaphorase [18] and the CN^--sensitive respiration detected by a Clark oxygen electrode. Similarly to the results of Table 3, the efficiency of the quinols in catalyzing electron transport through the quinol oxidase pathway was the following: Q_2H_2 ≫ 1,4-naphthoquinol > duroquinol ⋙ Q_0H_2 (data not shown). Assuming that the exogenously added quinols interact with the redox chain as a function of their thermodynamic features (see Ref. [17,18]), it is apparent that in light-grown membranes, the excess of a low-potential quinone such as rhodoquinone (E_m = −30 mV, RQ/UQ ≅ 0.9–1.7) does not seem to affect the efficiency of quinols with similar redox potentials in catalyzing the oxygen consumption.

Table 3
Light-activated quinol oxidase activities [a] by photosynthetic membranes from *Rf. fermentans* in the presence of different exogenously added quinones

Quinones	E'_o (mV)	Activity	% of control
1. –	–	0.200	100
2. Menadione	−1 [a]	0.208	104
3. Duroquinone	+35 [a]	0.200	100
4. 1,4-Naphthoquinone	+64 [a]	0.208	104
5. Decylubiquinone	+112 [b]	0.446	223
6. Q_0	+162 [a]	0.126	63

[a] Activities, expressed as μmol O_2 reduced h^{-1} (mg protein)$^{-1}$, were measured as described in Table 2 with succinate as substrate. The dark-control activity (succinate oxidation *plus* 10 μM CN^-) was 0.06 μmol O_2 reduced h^{-1} (mg protein)$^{-1}$. Each quinone was added to the final concentration of 150 μM.
Abbreviations used: Menadione, 2-methyl-1,4-benzoquinone; Duroquinone, tetramethyl-1,4-benzoquinone; Decylubiquinone, 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone; Q_0, 2,3-dimethoxy-5-methyl-1,4-benzoquinone.
[b] Values taken from Lemma et al. [18].
[c] Value taken from Clark [19].

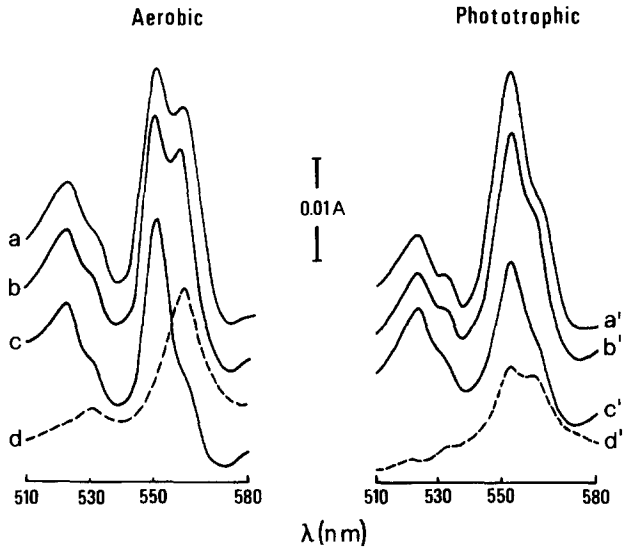

Fig. 3. Reduced *minus* oxidized difference spectra (recorded at 25° C) of whole-cell extracts and membrane fragments from *Rf. fermentans*. Samples suspended at 1.3 mg of proteins ml^{-1} in 50 mM Mops (pH 7.2) were oxidized with a few crystals of K− ferricyanide and reduced with a few crystals of sodium dithionite (a,a' and b,b') and ascorbate *plus* 10 μM DAD (c,c'). The interrupted traces represent the difference between the sodium dithionite- and ascorbate/DAD-reduced *minus* oxidized spectra.

3.3. Optical spectroscopy

Fig. 3 shows the spectra under different reducing conditions of the cytochromes detected in membranes and whole-cell extracts from light- and dark-grown cells. Traces a,b and a',b' (whole-cell extracts and membrane fragments, respectively) show peaks at 552 nm and 561 nm (α bands) which are indicative of *c*- and *b*-type cytochromes, respectively (see, however, Refs. [20] and [25]). Notably, a slight difference was seen between the amount of dithionite-reducible *c*-type cytochromes in whole-cell extracts (spectrum in a') and membrane fragments (spectrum in b') of light-grown cells with *c*/*b* molar ratios of 2.3 and 2.0, respectively. This suggests that a certain amount of periplasmic soluble *c* is lost during the isolation procedure of light-grown membranes. This conclusion was confirmed by the spectra of the soluble fractions (180 000 × *g*), indicating that approx 15 ± 1% and 1 ± 0.3% (mean of five preparations) of the total *c*-type cytochromes seen in

whole-cell extracts were released during fractionation of light- and dark-grown cells, respectively (not shown). Notably, the amount of soluble cytochrome *c* of light-grown *Rf. fermentans* (4.6 μmol of *c*-type haem (kg wet wt.)$^{-1}$) was similar to that observed in phototrophically grown *Rps. viridis* [21].

Spectra in c and c′ of Fig. 3 were recorded with ascorbate-DAD as reducing system. In aerobic membranes (c), the amount of *c*-type haem(s) reduced was 94% of that reducible with dithionite (trace b), whereas only 68% was reduced by ascorbate/DAD in light-grown membranes (c′) (see also the difference spectra, b − c = d and b′ − c′ = d′ [dotted traces]). This finding suggests that, although the cytochromes α and β bands of light- and dark-grown cells show similar values, their *c*-type content is likely to be qualitatively (besides quantitatively) different (see below).

The dark-potentiometric titration of the soluble fraction (180 000 × *g*) is shown in Fig. 4. The data at 550–540 nm (pH 7.0) show the presence of one component ($n = 1$) with E_m of +287 mV.

The dark potentiometric titrations of the cytochromes contained in membrane fragments of *Rf. fermentans* are shown in Fig. 5. Data obtained at 550 nm with 540 nm as reference (at pH 7.0) could be resolved into 4 and 3 components in light- and dark-grown membranes, respectively. The 4 haems of the photosynthetic membranes (1.2–1.3 nmol (mg protein)$^{-1}$) had midpoint potentials of +358, +296, +78 and −1 mV ($n = 1$) with relative contributions to the total absorbance signal of approx 23–25% for each component. Conversely, the 3 cytochromes *c* of aerobic membranes (0.8–0.9 nmol (mg protein)$^{-1}$) presented E_m values of +298, +201 and +44 mV with relative contributions of 47, 30 and 23% for

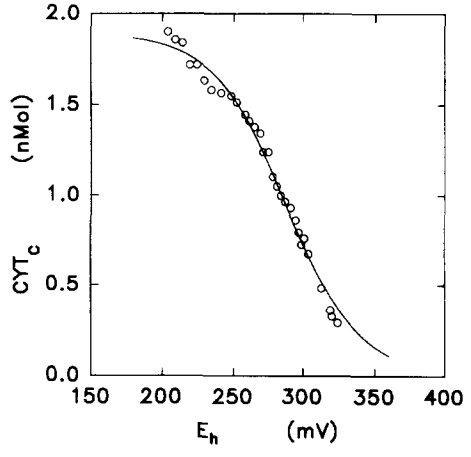

Fig. 4. Dark-equilibrium potentiometric titrations at 550–540 nm (pH 7.0) of the soluble-fraction (180000 × *g*) obtained from light-grown cells (see Materials and Methods). This fraction was 10-times concentrated by the use of an Amicon PM10 ultrafiltration membrane.

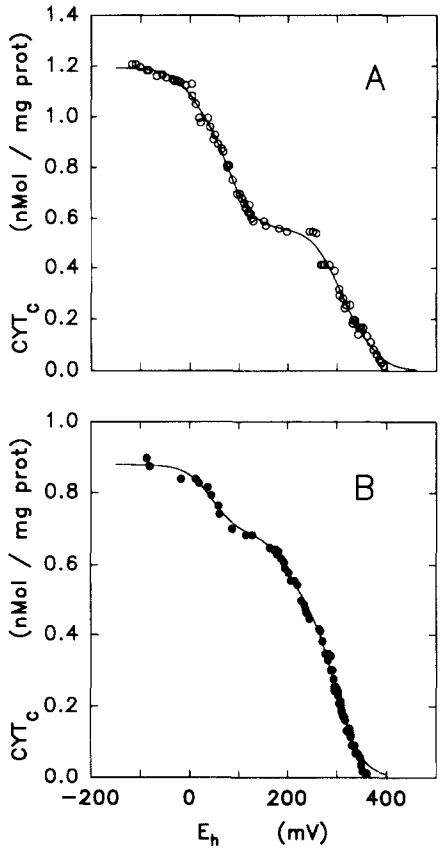

Fig. 5. Dark-equilibrium potentiometric titrations at 550–540 nm (pH 7.0) of light- (panel A) and dark-grown (panel B) membranes. See text for further details.

c-298, *c*-201 and *c*-44, respectively. These data indicate that: (i) the *c*-type content in light grown cells is ≅ 30% higher than aerobic grown cells on a molar/protein ratio (see also spectra of Fig. 2) and also (ii) there is no clear correlation between the E_m values of the *c*-type species of aerobic and photosynthetic membranes.

The dark-redox titrations of Fig. 6 show the $n = 1$ theoretical Nerst curves of the *b*-type haems detected at 560–575 nm in photosynthetic and aerobic membranes (curves A and B, respectively). The results of curve B could be fitted into a 4 components plot, to which were assigned E_m values of +371, +315, +76 and −18 mV. Conversely, the results of curve A show that while the midpoint potential part of the redox titration remains similar to its counterpart in B, the high-potential part is drastically changed, cytochrome *b* at +371 mV being absent. A second difference between curve A and B is that the two mid-low potentials cyt *b*'s at +76 and −18 mV of curve B are shifted to lower potentials in curve A (E_m of +30 and −50 mV). These data, when compared with those of Tables 1, 2 and 3, suggest that cyt *b*-371 might be involved in cyt *c* oxidase activity because it is absent from light-grown membranes in which the cyt *c* oxidase is barely detectable.

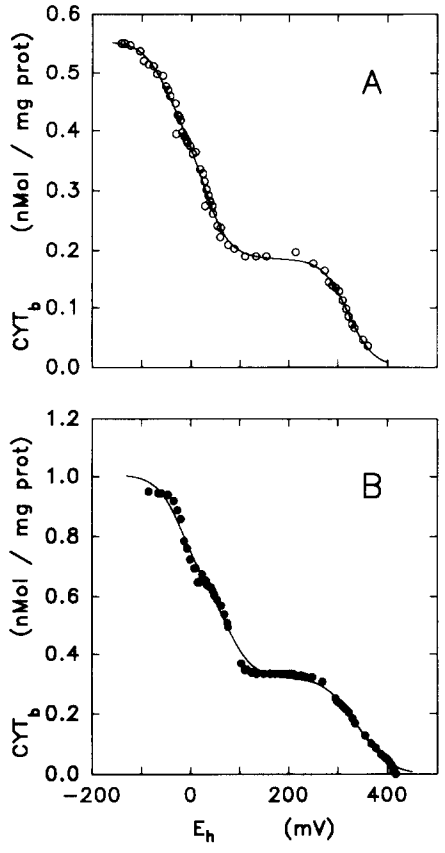

Fig. 6. Dark-equilibrium potentiometric titrations at 560–575 nm (pH 7.0) of light- (panel A) and dark-grown (panel B) membranes. See text for further details.

3.4. Oxido-reduction kinetics of cytochromes c

Traces of Fig. 7 represent the oxido-reduction kinetics of the *c*-type haems (550–540 nm) in aerobic- and photosynthetic membranes. It is apparent that in both types of membrane a large fraction (55–67%) of the total dithionite reducible *c*-haems is reduced by ascorbate-DAD under steady-state respiratory conditions. However, while in light-grown membranes the addition of 10 μM CN^- does not affect significantly the cyt *c*-reduction level (4% increase), the inhibition of the cyt *c* oxidase activity by CN^- in aerobic membranes raises the reduction level to 93%; this suggests that most of the *c*-type haems seen in aerobic membranes are in rapid equilibrium with the respiratory electron flow. Another significant difference between the two types of membrane, confirming the spectral data of Fig. 3, was that 37% of the cyts. *c* of light-grown membranes can only be reduced by dithionite (trace C). In addition, while 10 μM CN^- has no effect on the succinate-dependent steady-state reduction level of photosynthetic membranes (trace D), the same CN^- concentration had a strong effect on *c*-type reduction of aerobic membranes (traces A and B). Notably, the bc_1 inhibitor stigmatellin (see broken traces in B and D) slightly decreased (5%) the reduction level of the cyts. *c* in photosynthetic membranes (dashed trace in D), whereas it drastically lowered (from 21 to 2–5%) that of aerobic membranes (dashed trace in B). These data indicate that a very small fraction (10%) of the total amount of substrate reducible *c*-haems in photosynthetic membranes is involved in respiratory electron transport, whereas at least 50% of the cytochromes *c* seen at 550–540 nm in aerobic membranes (possibly cyt *c*-201 and cyt *c*-298) are rapidly oxidized by the cyt *c* oxidase.

4. Discussion and conclusions

Membranes from cells of the recently established new species of the facultative phototrophs, *Rhodoferax fermentans*, have been examined. It is concluded that *Rf. fermentans* is endowed with a respiratory chain composed by cytochromes of *c*- and *b*-type only. The present study also demonstrates that aerobically dark-grown *Rf. fermentans* does not contain soluble cytochromes of *c*-type. In addi-

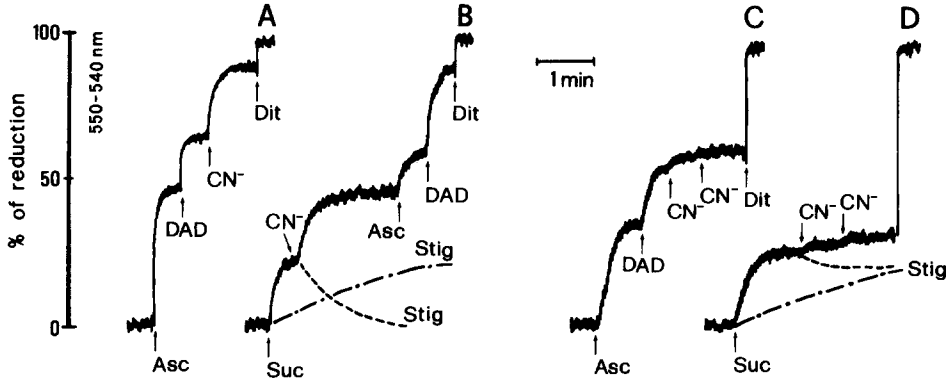

Fig. 7. Effects of cyanide and stigmatellin on the oxidation- reduction kinetics of cytochromes *c* detected at 550–540nm. Traces in A and B show reduction of cyt.(s) *c* by ascorbate (Asc)-DAD and succinate (Suc) in dark-grown membranes, whereas traces in C and D represent similar experiments in light-grown membranes. Additions: ascorbate, 5 mM; DAD, 10 μM; CN^-, 10 μM (1st addition); CN^-, 2 mM (2nd addition); dithionite (Dit), a few crystals; stigmatellin, 5 μM. In (——) stigmatellin was added upon the onset of the steady-state reduction level with succinate, whereas in (– · –) stigmatellin was present before addition of succinate.

tion, several important features of the electron transport chain(s) of this facultative phototroph have been established, namely: (a) the respiratory system is branched because two oxidative pathways can be distinguished by their CN^- sensitivities (shown in Fig. 1); (b) the branch must occur before the myxothiazol and/or stigmatellin sensitive sites because the NADH- and succinate-dependent respiration is only 50% blocked by these inhibitors; (c) the cytochrome *c* oxidase activity of phototrophically grown cells is poorly involved in respiratory electron transport because most of the oxygen consumption is catalyzed by the CN-resistant, i.e., quinol oxidase, pathway ($I_{50\%} = 2.5$ mM); (d) the quinol oxidase containing pathway is the one involved in light-dependent respiration because this activity is stimulated by inhibitors of the bc_1 complex (Table 2); (e) rhodoquinone, although present, is not involved in quinol oxidase activity because exogenously added quinones with $E_{m7.0} \leq +64$ mV do not stimulate the light-dependent respiration (shown in Table 3).

The thermodynamic analysis of membrane fragments from *Rf. fermentans* revealed a cytochrome *c*-type composition similar to that observed in species such as *Rhodopseudomonas viridis* and *Chloroflexus aurantiacus* [8] but quite different from *Rhodobacter capsulatus, Rhodospirillum rubrum* and *Rb. sphaeroides* [21–24]. Indeed, membranes from light-grown cells contain 4 cytochromes of *c*-type with E_m values of +358, +296, +78 and −1 mV which are present in a 1:1:1:1 (molar/protein) ratio. Although the thermodynamic data presented are not easily interpretable (see however [25]), some correlation between the tetrahaem *c*-type cytochrome of *Rps. viridis* and the *c*-type content of *Rf. fermentans* is clearly seen [26,27]. In addition, the soluble *c*-type component with α_{max} at 551 nm and $E_{m7.0}$ of +287 mV isolated from the soluble-fraction of light grown cells suggest that the *c*-type haem arrangement of *Rf. fermentans* might be analogous to that found in *Rps. viridis* [28] along with the fact that also the $E_{m7.0}$ of the RC of *Rf. fermentans*, +471 mV, is similar to that of *Rps. viridis* (see [25]). In line with the general observation that in facultative phototrophs the amounts of both the RC and of its primary electron donor [*c*-type haem(s)] are reduced in parallel by increasing the light-intensity and/or the oxygen tension [7], the cyt *c*/cyt *b* ratio (on a molar/protein basis) of *Rf. fermentans* decreases from 2.2 in light-grown membranes to 1.2 in dark-grown membranes, this variation being due to changes in the amounts of high-potential cyts *b* and *c* plus low-potential cyts *c* (compare Figs. 6 and 7).

An interesting, but still unexplained, result of this study concerns the 30–40 mV difference between the E_m values of the two mid-low potentials *b*-type haems titrated in both light-grown (E_m of +30 and −50 mV) and dark-grown (E_m of +76 and −18 mV) cells. By analogy with mitochondrial and bacterial electron transport chains, these two *b*-type haems are likely to be the protohaems (b_H and b_L) of complex III (bc_1) (see [8]). In this respect, thermodynamic considerations on the equilibrium between the quinone pool and the bc_1 complex [29] suggest the following correlation: $E_{m(Q\,pool)} = 1/2[E_{m(Fe-S)} + E_{m(b_L)}]$. This equation, when applied to the redox components titrated in *Rf. fermentans*, contains two unknown entities, namely: the E_m of the Q pool and that of the Rieske iron-sulfur centre (Fe-S). However, since aerobic membranes contain mainly UQ [6], assuming E_m values of +90 mV [29] and −18 mV (this work) for the UQ_{pool} and cyt b_L, respectively, the predicted E_m of the Rieske would be +198 mV. Since it is well established that cyt c_1 is in quasi-equilibrium with the Rieske centre [8], it is apparent that the cyt *c* titrated at +201 mV (see Fig. 5) is a good candidate for the role of cyt c_1 in *Rf. fermentans*.

Dark-grown membranes contain 3 *c*-type ($E_{m7.0}$ of +298, +201 and +44 mV) and 4 *b*-type cytochromes ($E_{m7.0}$ of +371, +315, +76 and −18 mV). Since cyt *b*-371 is lacking from light-grown membranes which also catalyze a negligible cyt *c* oxidase activity, it has been concluded that cyt *b*-371 is involved in this latter activity, although its physiological electron donor is not known. Indeed, the soluble fraction of aerobically grown cells does not contain significant amounts of soluble *c*-type haem ($\leq 1\%$ of the membrane-bound *c*-type collection); thus, a membrane-bound *c* (*c*-298?) is likely to reduce cyt *b*-371 in line with fact that a large fraction of the total substrate reducible *c*-haems is in rapid equilibrium with the respiratory electron flow (shown in Fig. 7).

The presence of high amounts of rhodoquinone (RQ/UQ ≅ 2) in light-grown *Rf. fermentans* is puzzling (see also Ref. [25]). We have recently established that the E_m of the primary quinone acceptor, Q_a, in this bacterial species is at +13 mV [20,25]. This suggests that Q_a is likely to be a ubiquinone molecule because the E_m of the RQ pool has been titrated at −30 mV [30]. It is therefore interesting to notice that an early study on a RQ-deficient mutant of *Rsp. rubrum* demonstrated that RQ was necessary for both photosynthetic growth and NADH-fumarate oxidoreductase activity [31,32]. This suggests that RQ might be required in maintaining the redox balance during photosynthetic growth of RQ-containing species such as *Rf. fermentans* and *Rsp. rubrum* through the use of a membrane-bound fumarate reductase. This activity, which is present in *Rf. fermentans* [4], would also involve the NADH dehydrogenase. These considerations might be relevant in explaining why piericidin, an inhibitor of complex I, is less effective in inhibiting NADH respiration of light-grown membranes than aerobic membranes. As a working hypothesis, one may indeed suggest that membranes of *Rf. fermentans* contain either two NADH dehydrogenases with different affinities for RQ and UQ or, by analogy with plant and yeast mitochondrial NADH dehydrogenases [33,34], one NADH dehydrogenase with two quinone interacting sites [35]. It is evident that further studies are necessary to discriminate between these work-

ing hypotheses and possibly clarify the role of rhodoquinone in anaerobically grown cells (dark and/or light) of *Rf. fermentans*.

Acknowledgements

A.H. was the recipient of a post-doctoral fellowship from the University of Bologna. Work supported by CNR (BTBS Program).

References

[1] Hiraishi, A. and Kitamura, H. (1984) Bull. Jpn. Soc. Sci. Fish 50, 1929–1937.
[2] Hiraishi, A. and Hoshino, Y. (1984) J. Gen. Appl. Microbiol. 30, 435–448.
[3] Hiraishi, A., Hoshino, Y. and Kitamura, H. (1984) J. Gen. Appl. Microbiol. 30, 197–210.
[4] Hiraishi, A. (1988) Arch. Microbiol. 150, 56–60.
[5] Hiraishi, A. (1988) FEMS Microbiol. Lett. 56, 199–202.
[6] Hiraishi, A., Hoshino, Y. and Satoh, T. (1991) Arch. Microbiol. 155, 330–336.
[7] Drews, G. and Oelze, J. (1981) Adv. Microbiol. Physiol. 22, 1–92.
[8] Amesz, I. and Knaff, D. (1988) in Biology of Anaerobic Microorganisms (Zehnder, A.J.B., ed.), pp. 113–178, John Wiley and Sons, New York.
[9] Zannoni, D., Jasper, P. and Marrs, B.L. (1978) Arch. Biochem. Biophys. 191, 625–631.
[10] Prince, R.C. and Daldal, F. (1987) Biochim. Biophys. Acta 894, 370–378.
[11] Zannoni, D. (1982) Biochim. Biophys. Acta 680, 1–7.
[12] Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
[13] Clayton, R.K. (1963) Biochim. Biophys. Acta 17:10–22.
[14] Yagi, T. (1991) J. Bioenerg. Biomembr. 23, 211–225.
[15] Zannoni, D. and Baccarini-Melandri, A. (1980) in Diversity of Bacterial Respiratory Systems (Knowles, K.J., ed.), Vol. II, pp. 183–202, CRC Press, Boca Raton.
[16] Ramirez-Ponce, M.P., Gimenez-Gallego, G. amd Ramirez, J.M. (1980) FEBS Lett. 114, 319–322.
[17] Zannoni, D. and Moore, A.L. (1990) FEBS Lett. 271, 123– 127.
[18] Lemma, E., Schagger, H. and Kroger, A. (1993) Arch. Microbiol. 159, 574–578.
[19] Clark, W.M. (1960) Oxidation-reduction potentials of organic systems. Williams & Wilkins, Baltimore.
[20] Hochkoeppler, A., Venturoli, G. and Zannoni, D. (1993) Biol. Chem. Hoppe-Seyler 374, 831.
[21] Bartsch, R.G. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 249–279, Plenum Press, New York.
[22] Venturoli, G., Fenoll, C. and Zannoni, D. (1987) Biochim. Biophys. Acta 892, 172–184.
[23] Zannoni, D., Venturoli, G. and Zannoni, D. (1992) Arch. Microbiol. 157, 367–374.
[24] Zannoni, D. and Daldal, F. (1993) Arch. Microbiol. 160, 413–423.
[25] Hochkoeppler, A., Venturoli, G. and Zannoni, D. (1995) Biochim. Biophys. Acta 1229, 81–88.
[26] Zannoni, D. and Ingledew, J.W. (1985) FEBS Lett. 193, 93–98.
[27] Zannoni, D. (1986) FEBS Lett. 198, 119–124.
[28] Garcia, D., Richaud, P. and Vermeglio, A. (1993) Biochim. Biophys. Acta 1144, 295–301.
[29] Meinhardt, S.W. and Crofts, A.R. (1983) Biochim. Biophys. Acta 723, 219.
[30] Erabi, T., Higuti, T., Kakuno, T., Yamashita, J., Tanaka, M. and Horio, T. (1975) J. Biochem. 78, 795–801.
[31] Gimenez-Gallego, G., Del Valle-Tascon, S. and Ramirez, J.M. (1976) Arch. Microbiol. 109, 119–125.
[32] Ramirez-Ponce, P., Ramirez, J.M. and Gimenez-Gallego, G. (1980) FEBS Lett. 119, 137–140.
[33] Moller, I.M. and Lin, W. (1986) Annu. Rev. Plant Physiol. 37, 309–334.
[34] Rugolo, M. and Zannoni, D. (1992) Plant Physiol. 99, 1037–1043.
[35] Soole, K.L., Dry, I.B. and Wiskich, J.T. (1990) Physiol. Plant. 80, 75–82.