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The relation between electron transfer, protonmotive force and lactose transport in membrane vesicles from aerobically grown *Rhodobacter sphaeroides* 4P1

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Membrane vesicles were isolated from *Rhodobacter sphaeroides* strain 4P1, grown aerobically in the dark. This strain is equipped with the lactose transport protein from *Escherichia coli*. In the membrane vesicles a branched electron-transfer chain is located; one branch contains cytochrome *c* oxidase and the other the so-called 'alternative oxidase'. Cytochrome *c* oxidase is the major energy-coupling device in these membrane vesicles. Several electron donors (succinate, NADH, reduced cytochrome *c*, etc.) are oxidized via the electron-transfer chain with different contributions of the two branches of the terminal part of the electron-transfer chain. The capacity of these membrane vesicles to generate a protonmotive force correlates well with the activity of the cytochrome *c* oxidase-containing branch of the electron-transfer chain. This correlation was revealed by the use of electron-transfer chain inhibitors like antimycin A and KCN. The rate and maximal level of lactose accumulation correlated chemiosmotically with the magnitude of the protonmotive force, assuming 1 H^+/lactose symport, without the need to invoke additional regulatory mechanisms. Electron donors that can directly reduce cytochrome *c* oxidase were most efficient in protonmotive force generation and in the energization of lactose transport.

Introduction

Rhodobacter sphaeroides is a facultative photoheterotrophic bacterium capable of growing either aerobically in the dark or anaerobically in the light. Aerobically in the dark a protonmotive force (Δp) is generated by a 'linear' electron transfer chain and anaerobically in the light by a cyclic (photosynthetic) electron-transfer chain [1,2]. Anaerobically in the dark, in the absence of electron transfer, *Rb. sphaeroides* can maintain a membrane potential of significant magnitude. Despite the presence of this membrane potential, no secondary transport of solutes occurs. Linear or cyclic electron transfer is necessary to initiate solute transport. It was postulated that the regulatory effect of electron transport on solute uptake is exerted via a redox control of the transport proteins by electron-transfer chain intermediates [3–6]. Konings and Robillard demonstrated that solute transport proteins in *Escherichia coli* membrane vesicles are redox-sensitive. A mechanism was proposed in which Δp controls the activity of transport proteins by dithiol/disulphide inter-

Abbreviations: $\Delta\psi$, transmembrane electric potential; ΔpH, transmembrane pH gradient; Δp, protonmotive force; ICM, intracellular membranes; TPP^+, tetraphenylphosphonium; BChl, bacteriochlorophyll; TMPD, *N,N,N′,N′*-tetramethyl-1,4-phenylenediamide hydrochloride; DTT, dithiothreitol; PMS, phenazine methosulphate.

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changes [7,8]. The molecular mechanism of this regulatory effect of electron transfer on solute uptake is still unknown.

Quantitative analysis of Δp, chemical gradient of solutes ($\Delta p_{solutes}$) and rates of secondary transport in *Rb. sphaeroides* and *E. coli* demonstrated that flow-force relations are affected by turnover rates of the electron-transfer chain [9]. We decided to investigate the effect of electron transfer on solute uptake in membrane vesicles of *Rb. sphaeroides* in which the membrane potential ($\Delta\psi$) can be accurately measured with the TPP^+ distribution method. Membrane vesicles were prepared from *Rb. sphaeroides* 4P1 which is equipped with the lactose transport protein via genetic manipulation [10]. Cells were grown under high oxygen tension in the dark. Under this condition bacteriochlorophyll synthesis and the formation of intracellular membranes (ICM) are severely repressed and *Rb. sphaeroides* contains a branched electron-transfer chain [11]. One branch has an alternative oxidase which is sensitive to high concentrations of KCN. The other branch resembles the respiratory chain of mitochondria and has an aa_3-type cytochrome *c* oxidase as the terminal oxidase [2,12]. The relation between linear electron transfer, Δp and lactose transport has been studied in membrane vesicles of *Rb. sphaeroides*. The results show that the terminal aa_3-type cytochrome *c* oxidase functions as the most important energy coupling site and that electron transfer via the alternative oxidase-containing branch does not result in the formation of a Δp. Furthermore, in membrane vesicles from *Rb. sphaeroides* 4P1, grown aerobically in the dark, lactose transport and Δp are coupled chemiosmotically.

Materials and Methods

Cell growth and preparation of membrane vesicles. *Rhodobacter sphaeroides* 4P1 (recently redefined from the genus *Rhodopseudomonas*) is a derivative of strain 2.4.1. (wild type) and harbours a plasmid which contains the *lac z* and *y* genes and a gene coding for streptomycin resistance under the control of an unknown *Rb. sphaeroides* promoter. *Rb. sphaeroides* 4P1 was constructed by Drs. S. Kaplan and F.E. Nano [10]. It was grown at 30 °C with vigorous aeration in the medium described by Sistrom [13] supplemented with streptomycin (50 μg/ml). Cells were harvested at the end of exponential growth at an optical density at 660 nm of 1.8–2.0. For transport measurements cells were washed twice with 50 mM potassium phosphate (pH 7.0) + 5 mM $MgSO_4$ and resuspended in this medium (10 mg protein/ml). Membrane vesicles were prepared as described by Kaback [14] resuspended in 50 mM potassium phosphate (pH 7.0) containing 5 mM $MgSO_4$ and stored in liquid nitrogen until use.

Transport studies in intact cells. Transport studies in cells under aerobic conditions were performed as described by Kaback [15]. Experiments under anaerobic conditions in the light were performed in Durham tubes in which, via a rubber stopper, water-saturated oxygen free nitrogen was blown, as described by Hellingwerf et al. [16].

Simultaneous measurements of lactose transport, $\Delta\psi$ and the rate of oxygen consumption in membrane vesicles. A polyvinylchloride vessel of 3 ml was used in which both a tetraphenyl-phosphonium (TPP^+) sensitive electrode and a Clark-type oxygen electrode were inserted, as described previously by Elferink et al. [17]. Experiments were performed at 30 °C. Samples (50 μl) were withdrawn from the incubation mixture with a Hamilton syringe at different time intervals. Samples were diluted with 2 ml ice-cold 100 mM LiCl, filtered on 0.45 μm cellulose nitrate filters and washed once with 2 ml ice-cold 100 mM LiCl. Dried filters were transferred to scintillation vials containing 4 ml scintillation fluid and radioactivity was measured with a liquid scintillation counter (Packard Tri-Carb-460 CD, Packard Instruments Comp.).

Calculations. The magnitude of the membrane potential was calculated with the Nernst equation. A correction for TPP^+ binding to the vesicles according to the model of Lolkema et al. [18] was applied. Steady state levels of lactose accumulation are expressed as Δp (*lac*) (mV) according to the relation $\Delta p\ (lac) = -60 \log [lac]_{in}/[lac]_{out}$, where $[lac]_{in}$ and $[lac]_{out}$ denote internal and external lactose concentrations, at steady-state levels of accumulation, respectively. For an uncharged solute like lactose, the phenomenological H^+/solute stoichiometry (n) can be calculated according to the relation: $n = \Delta p(lac)/\Delta p$ [19].

Analytical procedures. Protein was determined according to the method of Lowry et al. [20] with bovine serum albumin as a standard.

Materials. Horse heart cytochrome *c* and antimycin A were purchased from Sigma Chemicals Co. [D-glucose-1-^{14}C]lactose (57 mCi/mmol) and L-[^{14}C]-alanine (171 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, U.K. All other chemicals were reagent grade and obtained from commercial sources.

Results

Lactose transport in intact cells

Growth of *Rb. sphaeroides* 4P1 aerobically in the dark results in an almost complete repression of bacteriochlorophyll synthesis. The total bacteriochlorophyll content of these cells as measured by the method of Clayton [21] is about a 100-times lower (0.02 mg BChl/mg protein) than in cells grown anaerobically in the light. In accordance with these observations in these cells lactose accumulation under anaerobic-dark conditions was only slightly stimulated by light (Fig. 1). The uptake rates driven by endogenous respiration were significantly higher. The highest uptake rates (15 nmol lactose per min per mg protein) were observed under aerobic conditions in the presence of light or in the presence of the artificial electron donor ascorbate plus *N,N,N′,N′*-tetramethyl-1,4-phenylenediamide hydrochloride (TMPD).

Lactose uptake in membrane vesicles

To investigate in more detail the relation between electron transfer, protonmotive force (Δp) and lactose transport, membrane vesicles were prepared from *Rb. sphaeroides* 4P1 grown aerobically in the dark. The rate of uptake of lactose by these membrane vesicles in the absence of electron transfer was low. Under aerobic conditions upon addition of various electron donors the rate of uptake was markedly stimulated. Succinate could serve as an energy source and succinate together with TMPD or cytochrome *c* yielded even higher uptake rates (Fig. 2a). The artificial electron-donor systems ascorbate plus TMPD, ascorbate plus cytochrome *c* or ascorbate in the presence of both components could also effectively drive lactose transport (Fig. 2b). The oxidation of ascorbate

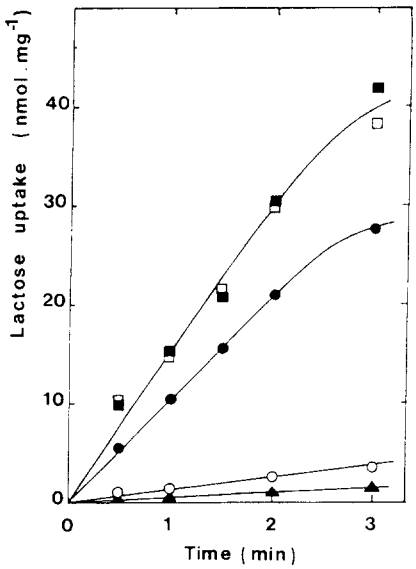

Fig. 1. Lactose uptake in cells of *Rb. sphaeroides* 4P1, grown aerobically in the dark. Cells were incubated anaerobically in the dark (▲); anaerobically in the light (○); aerobically in the light (□); aerobically in the dark in the absence and presence of ascorbate (20 mM) (●) and aerobically in the dark in the presence of ascorbate (20 mM) plus TMPD (200 μM) (■). [^{14}C]lactose was added to a final concentration of 197 μM.

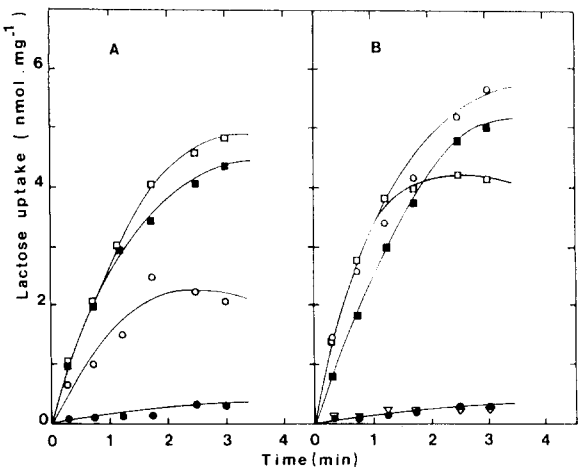

Fig. 2. Lactose uptake in membrane vesicles of aerobically grown *Rb. sphaeroides* 4P1. The incubation mixture contained 0.89 mg membrane protein/ml. 1 min after the addition of the electron donors [^{14}C]lactose was added to a final concentration of 197 μM. (A) No additions (●); succinate (○); succinate plus cytochrome *c* (■) and succinate plus TMPD (□). (B) No additions (●); ascorbate (△); ascorbate plus cytochrome *c* (■); ascorbate plus TMPD (□); ascorbate plus TMPD and cytochrome *c* (○). Final concentrations of succinate, ascorbate, TMPD and cytochrome *c* were 20 mM, 20 mM, 200 μM and 20 μM, respectively.

plus phenazine methosulphate (PMS) could energize lactose transport to the same extent as ascorbate plus TMPD (data not shown). Ascorbate alone did not stimulate lactose uptake above endogenous levels.

The relation between electron transfer, Δp and lactose transport in membrane vesicles

In the presence of the physiological electron donor NADH, no uptake of lactose above endogenous levels was observed. Although NADH was oxidized at a high rate no Δp was generated (Table I). When NADH was combined with TMPD, oxygen consumption was stimulated twofold. This resulted in the generation of a significant Δp and uptake of lactose. Also with succinate as electron donor, Δp formation and lactose accumulation were stimulated by TMPD or cytochrome *c*, although no further stimulation of oxygen consumption was observed. No further increase of Δp was observed when cytochrome *c* was added with NADH or succinate, in the presence of TMPD (data not shown). Addition of UQ_1 also stimulated lactose accumulation with NADH or succinate as electron donors. Oxidation of dithiothreitol (DTT) reduced UQ_1 resulted in lactose uptake to the same level as observed with NADH + TMPD (data not shown). Highest Δp values and lactose uptake rates were observed with ascorbate in combination with TMPD and cytochrome *c*. These results show that a poor correlation exists between the oxidation rate of the various electron donors and their ability to generate a Δp in membrane vesicles of *Rb. sphaeroides* 4P1.

The effects of electron-transfer chain inhibitors on respiration in membrane vesicles

The aerobic electron-transfer chain of *Rb. sphaeroides* is branched and one branch contains cytochrome *c* oxidase as the terminal oxidase [2]. To investigate the pathway for electrons from NADH and succinate to oxygen in the absence and presence of TMPD the effect of different electron-transfer chain inhibitors on the respiration rates was studied (Table II).

Antimycin A inhibited the rate of oxygen consumption in the presence of succinate and of NADH by 70% and by 60%, respectively. The oxidation of ascorbate plus TMPD was not inhibited. Succinate or NADH oxidation in the presence of antimycin A was not altered significantly when KCN was added to a final concentration of 100 μM. These results indicate that the antimycin-A-sensitive site is in the branch which has the cytochrome *c* oxidase as the terminal oxidase. In the presence of TMPD, antimycin A inhibited succinate and NADH oxidation by 44% and 25%, respectively. In this case a severe additional inhibition of succinate and NADH oxidation was observed when KCN (100 μM) was added (80% and 85%, respectively). The oxidation of

TABLE I

OXIDATION RATES OF VARIOUS ELECTRON DONORS, Δp GENERATION AND RATE OF LACTOSE UPTAKE BY MEMBRANE VESICLES OF *RHODOBACTER SPHAEROIDES* 4P1

Measurements were performed as described in Materials and Methods in the presence of 100 nM nigericin. [^{14}C]lactose was added to a final concentration of 197 μM. Cyt, cytochrome; n.d., not determined.

Substrate	O_2 consumption (nmol per min per mg)	Δp (mV)	$V_{lactose}$ (nmol per min per mg)	$\Delta p_{lactose}$ (mV)
Ascorbate [a]/TMPD/Cyt *c*	290	−65	4	−65
NADH	62	0	0	0
NADH/TMPD	124	−52	2.5	−63
NADH/Cyt *c*	n.d.	n.d.	2.4	−61
succinate	210	−43	1.5	−41
succinate/TMPD	210	−56	2.5	−62
succinate/Cyt *c*	200	−59	2.5	−60

[a] The concentrations of electron donors and mediators in the reaction mixture were: ascorbate/NADH/succinate, 20 mM; TMPD 200 μM and cytochrome *c*, 20 μM, respectively.

TABLE II

EFFECT OF ELECTRON-TRANSFER CHAIN INHIBITORS ON THE OXIDATION OF ELECTRON DONORS BY MEMBRANE VESICLES OF *RHODOBACTER SPHAEROIDES* 4P1

Oxygen consumption was measured in the absence and presence of electron-transfer chain inhibitors and is given as nmol/min per mg. Vesicles were preincubated with 100 μM antimycin A alone, or together with 100 μM KCN, for 5 min at room temperature. The respiration rates were corrected for autooxidation. The percentage inhibition in the presence of KCN was used to calculate the activity of the branch of the electron-transfer chain containing cytochrome *c*-oxidase. The values between brackets are given as % inhibition.

Substrate[a]	No addition	100 μM antimycin A	100 μM antimycin A +100 μM KCN	Cytochrome *c* oxidase activity
Succinate	210	63 (70)	63 (70)	147
Succinate/TMPD	210	118 (44)	42 (80)	168
NADH	72	29 (60)	28 (61)	44
NADH/TMPD	148	111 (25)	21 (85)	127
Ascorbate/TMPD	295	295 (0)	53 (82)	242

[a] Final concentrations of ascorbate, succinate, NADH were 20 mM and TMPD 200 μM, respectively.

ascorbate plus TMPD was inhibited to the same extent by KCN, irrespective of the presence or absence of antimycin A.

These results indicate that TMPD bypasses the antimycin-A-sensitive site and that electrons from succinate and NADH are donated to cytochrome *c* oxidase via TMPD. Therefore the rate of electron transfer via cytochrome *c*-oxidase in the presence of the various electron donors could now be estimated (Table II). When these rates of electron transfer from the various electron donors via cytochrome *c*-oxidase to oxygen were compared with their ability to drive lactose transport, an excellent correlation was found (Fig. 3). From these results it is concluded that cytochrome *c* oxidase is the most important energy coupling site in membrane vesicles from aerobically in the dark grown *Rb. sphaeroides* 4P1. To investigate whether electron flow via the alternative route could generate a Δp, oxygen consumption and Δp generation with succinate as electron donor were studied.

Succinate oxidation via the branched electron-transfer chain

In membrane vesicles succinate is oxidized at a high rate, which results in the formation of a $\Delta\psi$ as indicated by the uptake of TPP^+ (Fig. 4). KCN, at a concentration of 100 μM, inhibited respiration via the cytochrome *c* oxidase by more than 80% (Table II), but had no effect on succinate oxidation. It is most likely that succinate can be oxidized via both branches of the electron-transfer chain. When the cytochrome *c*-oxidase-containing

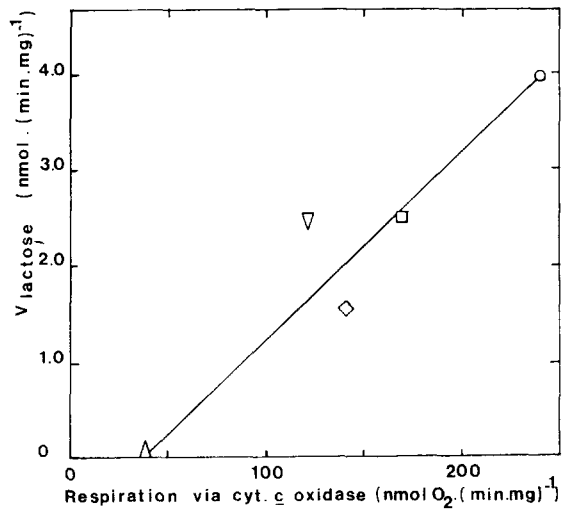

Fig. 3. The initial rate of lactose accumulation as a function of the respiration rate via the cytochrome *c*-oxidase-containing branch of the electron-transfer chain. The results given in Table II were used to calculate the respiration rate via the cytochrome *c* oxidase containing branch of the electron-transfer chain in the presence of various electron donors. The rates of lactose uptake from Table I were used. NADH (△); NADH plus TMPD (▽); succinate (◇); succinate plus TMPD (□); ascorbate plus TMPD plus cytochrome *c* (○).

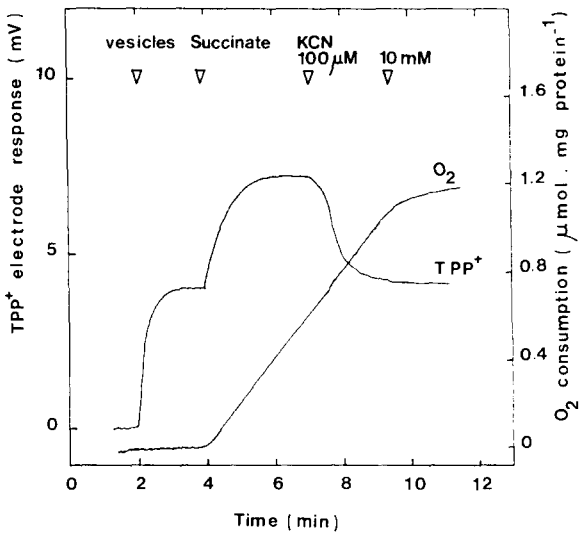

Fig. 4. Simultaneous recording of oxygen consumption and TPP^+ uptake in membrane vesicles of *Rb. sphaeroides* 4P1. As indicated vesicles were added to a final concentration of 0.98 mg/ml. The TPP^+ electrode response is due to dilution of the TPP^+ concentration after addition of the vesicles and to binding of TPP^+ to the vesicles. After the addition of succinate the effect of KCN on TPP^+ uptake and oxygen consumption were recorded. An increase in electrode potential reflects a decrease in TPP^+ concentration.

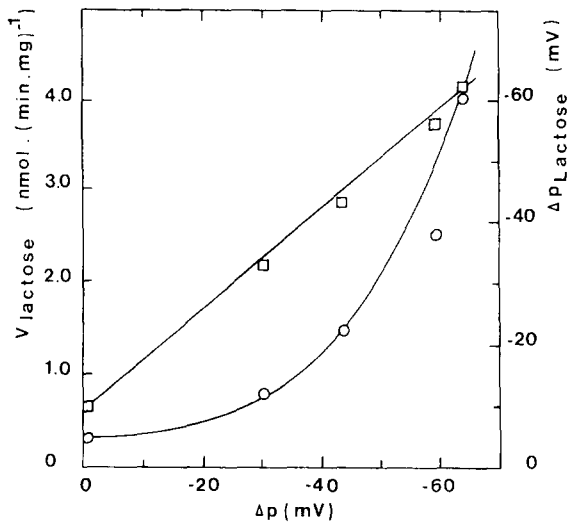

Fig. 5. The initial rate of lactose uptake and the steady-state level of lactose accumulation as a function of the Δp. Δp was varied by lowering the respiration rate of ascorbate (20 mM) plus TMPD (200 µM) with KCN. Vesicles were preincubated with different concentrations of KCN for 5 min at room temperature. KCN inhibited Δp generation via the cytochrome *c* oxidase with a K_i of 10 µM (data not shown). Nigericin (100 nM) was added to dissipate ΔpH so that Δp is composed of a $\Delta\psi$ only. $V_{lactose}$ (○) and $\Delta p_{lactose}$ (□).

branch is blocked, succinate oxidation continues with essentially the same rate via the alternative route. Under this condition succinate oxidation no longer results in the uptake of TPP^+. High concentrations of KCN (final concentration, 10 mM) inhibited succinate oxidation by more than 95%.

Lactose uptake and steady-state levels of accumulation as a function of the Δp

The (apparent) H^+/lactose stoichiometry (n) was determined from the lactose accumulation level (Δp lactose) and membrane potential ($\Delta\psi$ equals Δp under these conditions) as a function of the degree of inhibition of electron transfer with KCN. Lactose uptake ($V_{lactose}$) and steady-state accumulation levels ($\Delta p_{lactose}$) were differently dependent on the magnitude of the Δp (Fig. 5). The H^+/lactose stoichiometry in *Rb. sphaeroides* 4P1 membrane vesicles was approx. 1 (for calculation of n, see Materials and Methods) and remained constant upon variation of the Δp, indicating that lactose transport and Δp in membrane vesicles of *Rb. sphaeroides* 4P1 are coupled chemiosmotically.

Discussion

The results on Δp generation in membrane vesicles from *Rb. sphaeroides*, grown aerobically in the dark, obtained in this investigation can be combined with data available from the literature [22–29] into a tentative scheme as shown in Fig. 6. According to this scheme the electron-transfer chain is branched from the level of ubiquinone into one branch (I) which resembles the corresponding part of the mitochondrial electron-transport chain and in which 2 proton translocation sites are functional and into another branch (II), containing cytochrome *o* oxidase, in which electron transfer proceeds without the coupled proton translocation (Fig. 4). The two branches can be discriminated on the basis of their antimycin A and cyanide-sensitivity (Fig. 6). Input of electrons

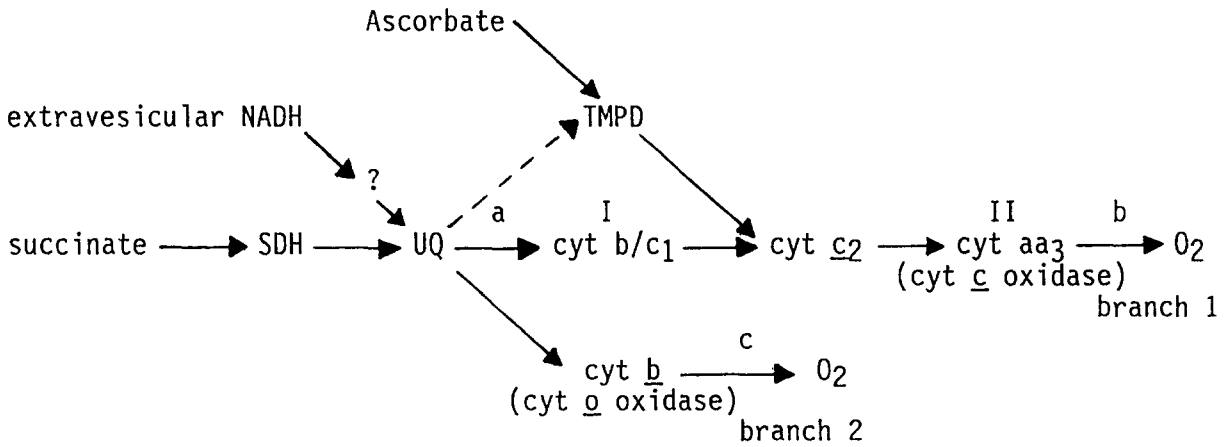

Fig. 6. Hypothetical scheme of the relevant part of the electron-transport chain in membrane vesicles from aerobically, dark grown *Rb. sphaeroides*. Roman numerals: sites at which proton translocation takes place. Letters: sites at which inhibitors act. a, antimycin A; b, KCN at 100 μM; and c, KCN at 10 mM. The question mark indicates that the mechanism of electron transfer from NADH to UQ is unknown.

into this chain has been achieved in this study in three ways: (i) with succinate, oxidized via the intravesicular succinate dehydrogenase [30]; (ii) with reduced TMPD (with excess ascorbate), leading to reduction of cytochrome *c*; and (iii) with NADH which, most probably, is not transported across the membrane of these vesicles and therefore donates electrons to the quinone pool [31,32,38]. This implies that in this report, Δp generation is limited to transfer of electrons via branch I. In intact cells an additional site for proton translocation is present between the NADH dehydrogenase and the quinone pool [2].

In isolated membrane vesicles electron transfer through branch I may be sub-optimal, due to loss of cytochrome c_2 upon isolation of the vesicles. This loss can be restored with exogenously added horse heart cytochrome *c* (Table I). Oxidized TMPD may cause a bypass of site I of proton translocation, as indicated by the dotted arrow in Fig. 6. In the unperturbed electron-transport chain electron transfer preferentially proceeds via branch I (Table II), in agreement with the results of Richaud et al. [23]. It is becoming more and more clear [26] that a scheme as shown in Fig. 6 can describe electron transport in many *Rhodospirillaceae*. One observation that is in conflict with this scheme was made by Zannoni [33] who observed that in a mutant of *Rb. capsulata* (M7) lacking the cytochrome *c* oxidase, the Q/cytochrome *b* region of the electron-transfer chain could still function as a proton translocation site.

These data on the relation between electron transfer and Δp generation can be used to rationalize the observations on lactose transport. A poor correlation was observed between the oxidation rates in general and the ability to energize lactose transport. However, a much better correlation exists between lactose transport (accumulation), and the Δp, generated under various conditions. This can now be understood, and indeed it is observed that a reasonably good correlation exists between the rate of electron transfer via branch I and the rate of lactose transport (Table II). This is to be expected in a chemiosmotic energy-transducing system in which back-pressure of the Δp on the rate of electron transfer is limited, due to a high endogenous leakage of the membrane [34].

Elferink et al. [35] presented evidence that the activity of the lactose transport protein when expressed in *Rb. sphaeroides* was regulated by the rate of electron transfer. Here, this regulation was not observed in membrane vesicles. The H^+/lactose stoichiometry was about 1, a value previously reported by others [19,36], although also values of larger than 1 have been reported [9,37]. The H^+/lactose stoichiometry in the membrane

vesicles was found to be 1 upon variation of the Δp, indicating that lactose transport and Δp are coupled chemiosmotically.

In contrast to the observation in whole cells [36] in membrane vesicles of *Rb. sphaeroides* in which initial rates of lactose uptake, steady-state accumulation levels and $\Delta\psi$ could be accurately measured no regulatory effect of electron transfer was observed. Recent experiments with cells of *Rb. sphaeroides* 4P1 indicate that the uptake of lactose and alanine is strongly dependent on the internal pH (Abee, T. et al., unpublished results).

Acknowledgements

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References

1 Baltscheffsky, M. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 595–614, Plenum Publishing Corp., New York.
2 Smith, L. and Pinder, D.B. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 641–656, Plenum Publishing, New York.
3 Elferink, M.G.L., Friedberg, I., Hellingwerf, K.J. and Konings, W.N. (1983) Eur. J. Biochem. 129, 583–587.
4 Elferink, M.G.L., Hellingwerf, K.J. and Konings, W.N. (1986) Biochim. Biophys. Acta 848, 58–68.
5 Elferink, M.G.L., Hellingwerf, K.J., Van Belkum, M.J., Poolman, B. and Konings, W.N. (1984) FEMS Microbiol. Lett. 21, 293–298.
6 Konings, W.N., Driessen, A.J.M., Elferink, M.G.L. and Poolman, B. (1987) in: Membrane Receptors, Dynamics and Energetics (Wirtz, U.W.A., ed.), pp. 237–251, Plenum Press, New York.
7 Robillard, G.T. and Konings, W.N. (1982) Biochemistry 20, 5025–5032.
8 Konings, W.N. and Robillard, G.T. (1982) Proc. Natl. Acad. Sci. USA 79, 5480–5484.
9 Hellingwerf, K.J., Van der Rest, M.E. and Bult, R. (1986) EBEC Short Reports, vol. 4, 294.
10 Nano, F.E. (1984) Ph.D. Thesis, University of Illinois, Urbana, IL.
11 Chorey, J.C., Donohue, T.J., Varga, A.R., Staehelin, L.A. and Kaplan, S. (1984) J. Bact. 159, 540–554.
12 Gennis, R.B., Casey, R.P., Azzi, A. and Ludwig, B. (1982) Eur. J. Biochem. 125, 189–195.
13 Sistrom, W.R. (1960) J. Gen. Microbiol. 22, 778–785.
14 Kaback, H.R. (1974) Methods Enzymol. 31, 698–709.
15 Kaback, H.R. (1971) Methods Enzymol. 22, 99–120.
16 Hellingwerf, K.J., Michels, P.A.M., Dorpema, J.W. and Konings, W.N. (1975) Eur. J. Biochem. 55, 397–406.
17 Elferink, M.G.L. (1986) Ph.D. Thesis, University of Groningen, Groningen.
18 Lolkema, J.S., Hellingwerf, K.J. and Konings, W.N. (1982) Biochim. Biophys. Acta 681, 85–94.
19 Booth, I.R., Mitchell, W.J., Hamilton, W.A. (1979) Biochem. J. 182, 687–696.
20 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
21 Clayton, R.K. (1963) Biochim. Biophys. Acta 75, 321–323.
22 Takamiya, K. (1984) Plant Cell Physiol. 25(5), 769–774.
23 Richaud, P., Marrs, B.L. and Verméglio, A. (1986) Biochim. Biophys. Acta 850, 216–260.
24 Armitage, J.P., Ingham, C. and Evans, M.C.W. (1985) J. B. 161, 967–872.
25 Matsushita, K., Yamada, M., Shinagawa, E., Adachi, O. and Ameyama, M. (1983) J. Biochem. 93, 1137–1144.
26 Fenoll, C. and Ramírez, J.M. (1984) Arch. Microbiol. 137, 42–46.
27 Hudig, H., Kaufmann, N. and Drews, G. (1986) Arch. Microbiol. 145, 378–385.
28 Zannoni, D., (1984) Arch. Microbiol. 140, 15–20.
29 Zannoni, D., Peterson, S. and Marrs, B.L. (1986) Arch. Microbiol. 144, 375–380.
30 Elferink, M.G.L., Hellingwerf, K.J., Michels, P.A.M., Seijen, H.G. and Konings, W.N. (1979) FEBS Lett. 107, 300–307.
31 Owen, P. and Kaback, H.R. (1979) Biochemistry 18, 1413–1422.
32 Bergsma, J. (1983) Ph.D. Thesis, University of Groningen, Groningen.
33 Zannoni, D. and Baccarini-Melandri, A. (1980) In Diversity of Bacterial Respiratory Systems (Knowles, C.J., ed.), Vol. 2, pp. 183–202, CRC Press, Boca Raton, FL.
34 Pietrobon, D. and Caplan, S.R. (1986) Biochemistry 25, 7682–7690.
35 Elferink, M.G.L., Hellingwerf, K.J., Nano, F.E., Kaplan, S. and Konings, W.N. (1983) FEBS Lett. 164, 185–190.
36 Matsushita, K., Patel, L., Gennis, R.P. and Kaback, H.R. (1983) Proc. Natl. Acad. Sci. USA 80, 4889–4893.
37 Konings, W.N., Ten Brink, B., Lolkema, J.S., Hellingwerf, K.J. (1981) in: Vectorial Reactions in Electron and Ion Transport in Mitochondria and Bacteria (Palmieri, F., Quagliariello, E., Siliprandi, N. and Slater, E.C., eds.), pp. 323–327, Elsevier/North-Holland/Biomedical Press, Amsterdam.
38 Zannoni, D., Peterson, S. and Marrs, B.L. (1986) Arch. Microbiol. 144, 375–380.