Biochimica et Biophysica Acta, 1015 (1990) 189–194
Elsevier

BBABIO 43116

Comparison of the contribution from different energy-linked reactions to the function of a membrane potential in photosynthetic bacteria

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(Received 15 June 1989)
(Revised manuscript received 25 August 1989)

Key words: Bacterial bioenergetics; Membrane potential; (*R. rubrum*); (*Rps. viridis*)

The steady-state membrane potentials generated by light, PP_i, ATP or the reverse transhydrogenase reaction were studied in chromatophores from two different phototrophic bacteria, *Rhodospirillum rubrum* and *Rhodopseudomonas viridis*. The membrane potentials generated by the different energy-linked reactions were evaluated by a tetraphenylboron (TPB^-) ion-selective electrode. The $\Delta\Psi$ generated by light was estimated to be 110 mV and 50 mV in *R. rubrum* and *Rps. viridis* chromatophores, respectively. In the dark, PP_i, ATP and reversed transhydrogenase generated membrane potentials in *R. rubrum* and *Rps. viridis* chromatophores 50, 60 and 35 mV, and 14, 35 and 25 mV, respectively. The effect of magnesium ion on the membrane potential generated by different energy-linked reactions was also studied. The $\Delta\Psi$ induced by different energy-generating reactions in *R. rubrum* and *Rps. viridis* chromatophores and the possible relationship to the chromatophore structures are discussed.

Introduction

The proton electrochemical potential ($\Delta\tilde{\mu}_{H^+}$) difference across the coupled plasma membrane in phototrophic bacteria plays an essential role in (photo) oxidative phosphorylation and active transport, and is composed of both a concentration part (ΔpH) and an electrical potential part ($\Delta\Psi$) [1,2]. The exact measurements of these quantities are required for studies of the mechanism of energy transduction. It was shown that *R. rubrum* chromatophores, associated with a phospholipid-impregnated filter, generated a photoelectric current upon oxidation of NADH by oxygen, by reverse transhydrogenase reaction and by ATP or PP_i hydrolysis [3–5]. The purified enzymes H^+-ATPase, H^+-PPase and transhydrogenase were shown to act as energy generators (for reviews see Refs. 6, 7). In chromatophores from *Rps. viridis* energy-linked behavior was also induced by addition of PP_i or ATP [8]. Recently, it was shown that chromatophores isolated from *Rps. viridis* have a membrane-bound PP_i synthesis activity coupled to cyclic electron transport [9].

Ion-selective electrodes for the evaluation of both $\Delta\Psi$ and ΔpH have been developed by Kamo et al. [10] and Setty et al. [11], respectively. The electrode responds selectively to $\Delta\Psi$ with TPP^+ [10–12] and TPB^- [13] ion concentration changes. These electrodes are widely used in membrane bioenergetics to measure the membrane potential of plasma membranes and chromatophores [10–13].

In this paper we report the magnitude of the membrane potential generated from different energy-linked reactions in chromatophores from *R. rubrum* and *Rps. viridis*.

Materials and Methods

Preparation of chromatophores

R. rubrum (S1) [14], and *Rps. viridis* (DSM133) [9] were grown anaerobically in light at 30 °C. Cells were harvested and chromatophores were prepared as in Ref. 16, except that cells from *Rps. viridis* were disrupted by two passages through a French pressure cell fractiona-

Abbreviations: BChl, bacteriochlorophyll; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; F_0F_1-ATPase, membrane-bound ATP synthetase; H^+-PPase, membrane-bound proton translocating PP_i synthase; $\Delta\Psi$, transmembrane electrical potential difference; TPB^-, tetraphenylborate ion; TPP^+, tetraphenylphosphonium ion; $\Delta\tilde{\mu}_{H^+}$, electrochemical gradient; S-13, 2′,5-Dichloro-3-*tert*-butyl-4′-nitrosalicylanilide.

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tor. Bacteriochlorophyll was measured by using the in vivo extinction coefficient of $\epsilon_{880nm} = 140\ mM^{-1} \cdot cm^{-1}$ and by extraction with acetone/methanol (7:2, v/v) using $\epsilon_{790nm} = 75\ mM^{-1} \cdot cm^{-1}$ for *R. rubrum and Rps. viridis*, respectively [15].

Electron micrograph

The chromatophores prepared from *Rps. viridis* were taken on the Philips EM 400T electron microscope, recorded at 80 kV. For negative staining, samples were placed on a collodion carbon-coated copper grid and stained with 2% uranyl acetate (pH 7.2) for 30 s. Excess fluid was removed with filter paper and the grid was left at room temperature to dry for 30 min.

Membrane potential measurements

The $\Delta\Psi$ (interior positive) was calculated from the distribution of TPB^- between the bulk phase and the intravesicular phase using the Nernst equation. The intravesicular concentration was calculated from the amount of TPB^- which had disappeared from the external medium with an ion-selective electrode [10–13], and an attempt was made to correct for the unspecific TPB^- accumulation, according to the model of Lolkema et al. [17]. The internal osmotic volume of the chromatophores was taken to be 1 μl per 20 nmol BChl [18].

The experiments were carried out in a 5 ml vessel containing 2 ml of assay buffer at constant temperature, 23°C. The assay medium contained: 1 μM TPB^-; 0.1 μM TPP^+ (to increase the permeability of the chromatophore membrane for TPB^-); 50 mM sodium glycylglycine (pH 7.5) and 25 mM NaCl. Magnesium acetate (5 mM) and sodium succinate (0.1 mM) were added to the assay unless otherwise stated. Chromatophores were added to the assay in a small volume to give a final concentration corresponding to 10–15 μM BChl. The reaction was started either by illumination or by addition of 0.5 mM ATP; 0.5 mM PP_i; 1 mM NADH; 0.5 mM NADPH plus 0.5 mM NAD^+ and 0.1 mM succinate separately. The actinic light intensity was 1.5 W/m^2. FCCP was purchased from Sigma (St. Louis, U.S.A.). Myxothiazol and stigmatellin were kindly donated by Dr. H. Reichenbach (Gesellschaft für Biotechnologische Forschung, Braunschweig, F.R.G.). TPP^+Cl^- and Na^+TPB^- were from Aldrich (Steinheim, F.R.G.). Other materials were of reagent grade and were obtained from commercial sources.

Results and Discussions

Light-induced membrane potential

Fig. 1 illustrates a typical trace of the light-driven membrane potential generation in *R. rubrum* and *R. viridis* chromatophores. In *R. rubrum* chromatophores the size of the membrane potential ($\Delta\Psi$) generated by light-driven cyclic electron transport was 110 mV (Fig. 1), whereas the corresponding $\Delta\Psi$-induced in *Rps. viridis* chromatophores was only 50 mV (Fig. 1). Different light-induced $\Delta\Psi$ values were observed in chromatophores by different methods, ranging from 60 to 140 mV (Ref. 13; see Refs. 18, 19). There are no data on $\Delta\Psi$ generated by *Rps. viridis* chromatophores in the literature. The unspecific binding of TPB^- ions to chromatophore membranes from *Rps. viridis* was much higher, corresponding to an electrode response of 50–65 mV as opposed to from *R. rubrum* chromatophores 15–25 mV (not shown). The $\Delta\Psi$ calculations after correction [17] were not affected by the magnitude of the unspecific binding. The light-induced membrane potential was in both kinds of chromatophore sensitive to inhibitors of the cyclic-electron transport and to uncouplers (Table I). *ortho*-Phenanthroline, an inhibitor of the reaction center, also blocked light-induced $\Delta\Psi$ (Table I). The $\Delta\Psi$ formation was in both types of chromatophore not sensitive to oligomycin, NaF or rotenone (Table I). In the presence of nigericin the $\Delta\Psi$ generated by light was stimulated by 140% and 200% in *Rps. viridis* and *R. rubrum* chromatophores, respectively, but in the presence of 1 μM valinomycin the $\Delta\Psi$ induced was totally inhibited (not shown).

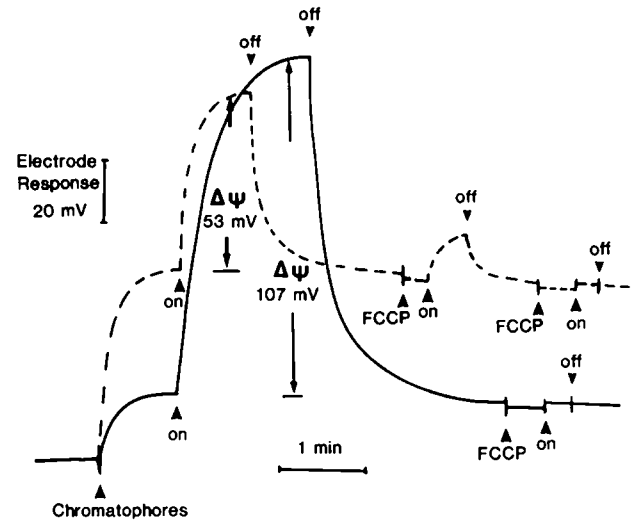

Fig. 1. A typical trace of the light-induced $\Delta\Psi$ in *R. rubrum* (12 μM) (———) and *Rps. viridis* (10 μM) (- - - - -) chromatophores. The assay mixture was described under the Materials and Methods. FCCP additions were 1 μM BChl.

The NADH- and succinate-induced $\Delta\Psi$ by non-cyclic dark electron transport were also investigated. The NADH and the succinate oxidation induced $\Delta\Psi$ to a very low extent in both kinds of chromatophore (Table II). Low generation of $\Delta\Psi$ by NADH and succinate oxidation reflects the anaerobic growth of the cells used for the chromatophore preparations.

PP_i-induced membrane potential

The membrane-bound pyrophosphatase, coupled to the electron transport chain, was found some time ago

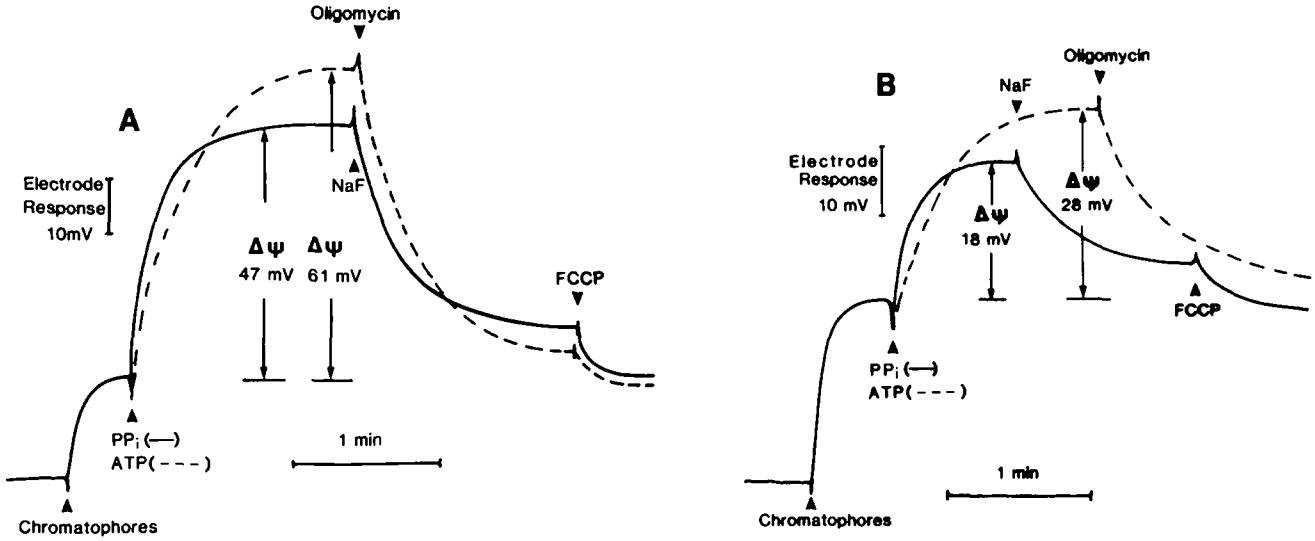

Fig. 2. A typical trace of ATP of PP_i-induced $\Delta\Psi$ generation in (A), *R. rubrum* (13.5 μM) and (B), *Rps. viridis* (12 μM) chromatophores. The reaction was started by addition of 0.5 mM ATP (- - -) and 0.5 mM PP_i (———) as described under the Materials and Methods. Oligomycin (5 μg/ml) and NaF (10 mM) were added as indicated.

in *R. rubrum* chromatophores (Ref. 20 and see Ref. 7) and also recently in *Rps. viridis* chromatophores [9]. This enzyme is unique in that it catalyzes not only the hydrolysis of PP_i, but also the synthesis of PP_i in the light [9,21–23]. It has been shown previously that PP_i hydrolysis is coupled to different energy transduction reactions in *R. rubrum* chromatophores (see Refs. 7, 24). In this study the absolute value of the membrane potential formed upon PP_i hydrolysis was measured. The addition of 0.5 mM PP_i to a suspension containing *R. rubrum* chromatophores induces a membrane potential of about 47 mV (Fig. 2a). The corresponding value of *Rps. viridis* chromatophores was only 18 mV (Fig. 2b). The potential generated by PPi in *R. rubrum* was 2–3-times higher than the $\Delta\Psi$ generated from *Rps. viridis*. One explanation for this result is the lower PPase activity in *Rps. viridis* [9].

The membrane-bound pyrophosphatases [25,26] require Mg^{2+} for the enzyme activity and the Mg^{2+}-PP_i complex is the functional substrate. The membrane-bound enzyme has a very poor hydrolysis activity with any other divalent cation or monovalent cations [25,26]. We observed that the PP_i-induced $\Delta\Psi$ was dependent on Mg^{2+} concentration (Fig. 3a). No $\Delta\Psi$ was induced by PP_i at Mg^{2+} concentrations lower than 0.3 mM (Fig. 3a). This effect is due to the very low concentration of Mg^{2+}-PP_i complex (lower than the K_m value) [25]. At constant concentration of PP_i (0.5 mM), an excess amount of free Mg^{2+} up to 5 mM was not inhibitory, either for the PP_i-induced potential response (Fig. 3a) or

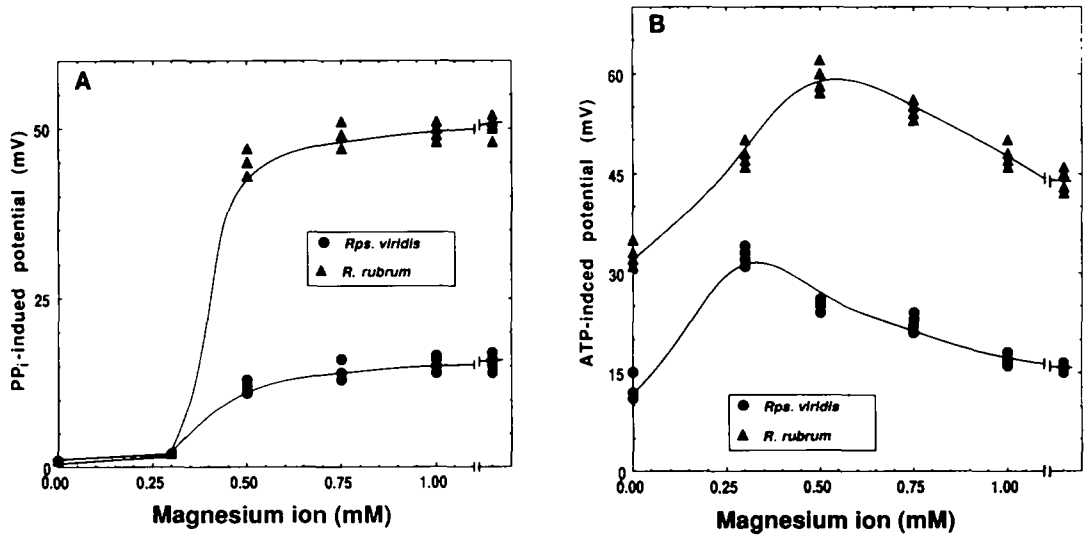

Fig. 3. The PP_i-induced (A) and ATP-induced (B) $\Delta\Psi$ generation as a function of the Mg^{2+} concentration in *R. rubrum* (▲) and *Rps. viridis* (■) chromatophores.

TABLE I

Effect of different inhibitors on the light-induced ΔΨ

The assay medium was described in the Materials and Methods. 100% corresponds to a ΔΨ of 110 mV and 50 mV for *R. rubrum* and *Rps. viridis* chromatophores, respectively.

Inhibitor		Light-induced ΔΨ(%)	
		R. rubrum	*Rps. viridis*
None		100	100
Myxothiazol	0.5 μM	15	25
Stigmatellin	1 μM	5	10
o-Phenanthroline	1 mM	0	0
Rotenone	5 μM	95	100
NaF	10 mM	92	93
Oligomycin	5 μg/ml	96	100
Valinomycin	5 μM	0	0
FCCP	1 μM	10	40
	3 μM	0	0

to the PPase activity. The potential achieved at 5 mM Mg^{2+} ion concentration is about 33% and 45% of the light-induced potential in *Rps. viridis* and *R. rubrum*, respectively. The PP_i-induced ΔΨ could be blocked in the presence of the PPase inhibitor, fluoride (10 mM) and by the uncouplers FCCP, S-13 and gramicidin. The ΔΨ generated was not sensitive toward electron transport inhibitors or oligomycin (not shown).

ATP-induced membrane potential

The ΔΨ generated by ATP hydrolysis is higher than the ΔΨ generated by PP_i hydrolysis in both *R. rubrum* and *Rps. viridis* chromatophores (Fig. 2a, b). This is in contrast with previous works, where the relative ΔΨ induced by PP_i was higher than the ATP-induced potential [3–5,27,28]. All those experiments were performed in the presence of a high concentration of Mg^{2+} when the ATP-induced potential rate was partially inhibited (Fig. 3b). The ATP-induced potential in *Rps. viridis* and *Rps. rubrum* chromatophores was 75% and 55% of the light, respectively. The ATP-induced ΔΨ in *R. rubrum* and *Rps. viridis* chromatophores is regulated by the addition of Mg^{2+} ions (Fig. 3b). The optimal ATP-induced potential was 30 mV at 0.3 mM Mg^{2+} and 60 mV at 0.5 mM Mg^{2+} in *Rps. viridis* and *R. rubrum* chromatophores respectively (Fig. 3b). In the absence of Mg^{2+} ions, the ATP-induced ΔΨ was totally inhibited by addition of 0.3 mM EDTA. This effect is probably due to endogenous divalent cations. It is shown also that the ATP hydrolysis and synthesis is fully dependent on addition of divalent cations of the EDTA-washed chromatophores [29].

Addition of 0.5 mM PP_i plus 0.5 mM ATP simultaneously to the chromatophore suspension induced a higher potential than if ATP or PP_i were added separately (Table II). The total potential induced by ATP and PP_i together was 75–80 mV and 30–35 mV at 5 mM Mg^{2+} in the chromatophores isolated from *R. rubrum* and *Rps. viridis* respectively (Table II).

TABLE II

Comparison of ΔΨ induced by different energy-linked reactions

The optimal ΔΨ induced was measured as described in the Material and Methods. The absolute ΔΨ were mean values from six experiments

Energy source	ΔΨ (mV)	
	R. rubrum	*Rps. viridis*
Light	110 (100%)	50 (100%)
PP_i	50 (45%)	15 (30%)
ATP	60 (55%)	30 (60%)
ATP + PP_i	75 (68%)	35 (70%)
Transhydrogenase	33 (30%)	25 (50%)
NADH	15 (14%)	5 (10%)
Succinate	10 (9%)	3 (6%)

Reverse transhydrogenase-induced membrane potential

In photosynthetic bacteria, the membrane-bound transhydrogenase is chemiosmotically coupled to cyclic electron transport [3,30,31]. The reversed transhydrogenase reaction can drive both PP_i synthesis and ATP synthesis in *R. rubrum* chromatophores [32]. *Rps. viridis* chromatophores also has an active transhydrogenase which is also coupled to cyclic electron transport [8,33]. The ΔΨ induced by reverse transhydrogenase activity were 33 mV and 25 mV in *R. rubrum* and *Rps. viridis* chromatophores respectively (Table II). The ΔΨ was 50% and 30% of the light-induced ΔΨ in *Rps. viridis* and *R. rubrum* chromatophores, respectively. The reverse transhydrogenase driven ΔΨ was sensitive to FCCP and the products of the reversed transhydrogenase reaction $NADP^+$ or NADH, but not to electron transport inhibitors (not shown).

Conclusion

The data in Table II summarize the contribution of the membrane potential generated by different energy-linked reactions. In all instances the potential induced in *R. rubrum* chromatophores is higher than that in *Rps. viridis* chromatophores. There are two major explanations for that difference: first, the chromatophore membranes of *Rps. viridis* are not as well coupled as the chromatophore membranes of *R. rubrum*; second, the synthesis as well as the hydrolysis activities of ATP and PP_i are much lower in *Rps. viridis* compared to *R. rubrum* chromatophores [9]. The major variation may be due to the difference in the membrane organization in these two phototrophic bacteria. We found that the membrane organization of *Rps. viridis* chromatophores consists of a double-layered (lammelle) membrane as is shown by electron micrograph (Fig. 4). It seems that the

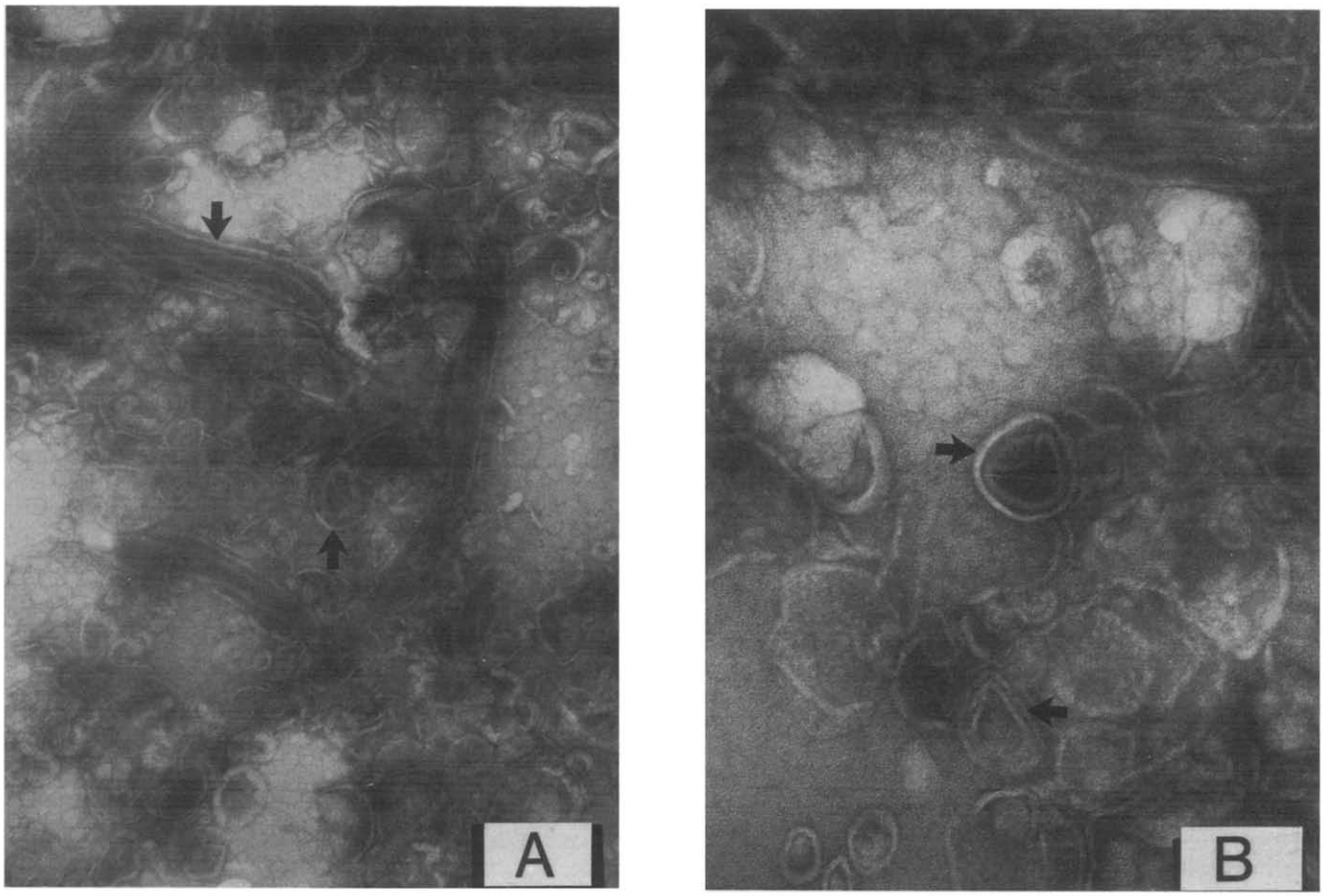

Fig. 4. Negative staining of electron micrograph of isolated chromatophores of *Rps. viridis* with a magnification ×60000 (A) and ×100000 (B).

majority of plasma membranes from *Rps. viridis* cells form heterogeneous vesicles, rather than homogeneous, single-layered membrane vesicles as in the *R. rubrum* chromatophores. Therefore it is possible that the *R. viridis* membrane is the rate limiting stage in the potential generation across the double-layered membrane.

The data show the contribution of potentials induced from PP_i and ATP hydrolysis and the reverse transhydrogenase reaction in two different photosynthetic bacteria. These energy-linked processes, over the $\Delta\tilde{\mu}_{H^+}$, may well regulate the energy balance and maintain an optimal energy economy under stress conditions.

Acknowledgements

This work was supported by a grant from the Swedish Natural Science Research Council to M.B. We thank Dr. Salam Al-Karadaghi from the Department of Structural Chemistry, University of Stockholm, for help with electron microscopy.

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