Electrochemical Proton Gradient across the Cell Membrane of *Halobacterium halobium*: Comparison of the Light-Induced Increase with the Increase of Intracellular Adenosine Triphosphate under Steady-State Illumination†

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ABSTRACT: The increase of the proton motive force mediated by bacteriorhodopsin is compared to the intracellular ATP concentration under steady-state illumination. The membrane potential was measured via the accumulation of the lipophilic ion [^{14}C]triphenylmethylphosphonium and the pH gradient via the accumulation of the weak acid 5,5-dimethyloxazolidine-2,4-dione. Light causes a parallel increase of ATP level and membrane potential at an external pH of 8. In contrast, at pH 6 an increase of the intracellular ATP concentration occurs without a corresponding increase of the proton motive force. If the extracellular NaCl concentration is reduced and not replaced by other ions, no membrane potential at all can be measured at pH 6–7; nonetheless, light-induced ATP synthesis occurs. A significant enhancement of the pH gradient occurs only at irradiances higher than those required for the attainment of the maximal intracellular ATP concentration. In the presence of the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone it is possible to obtain light-induced ATP synthesis without a measurable proton motive force in basal salt at pH 8. It is concluded that if bacteriorhodopsin acts as a proton pump, then the pumped protons can be used for ATP synthesis before they equilibrate with the protons in the extracellular bulk phase.

It is generally accepted that bacteriorhodopsin in the cell membrane of the halobacteria acts as a light energy converter [for review, see Henderson (1977)]. There is excellent evidence that bacteriorhodopsin pumps protons from the cell interior into the extracellular medium. The result is that an electrochemical potential difference develops across the cell membrane. The proton motive force, $\Delta\bar{\mu}_{H^+}/F$,[1] is given by

$$\frac{\Delta\bar{\mu}_{H^+}}{F} = \Delta\psi - \frac{2.3RT}{F}\Delta\mathrm{pH} \qquad (1)$$

where $\Delta\psi$ is the electrical potential difference across the membrane, the membrane potential, and ΔpH is the pH difference. According to the chemiosmotic hypothesis (Mitchell, 1966) the membrane potential can drive the uptake of cations, and even of neutral molecules and anions, if their influx is coupled to the influx of cations. The total electrochemical potential difference of the proton can be used for ATP synthesis by a vectorial proton-translocating ATPase. If there is a fixed stoichiometry between the number of protons translocated per molecule of ATP synthesized or hydrolyzed, a thermodynamic relationship should exist between the electrochemical proton gradient and the intracellular ATP, ADP, and P_i concentrations under steady-state conditions. Under these conditions, the rate of ATP synthesis driven by the electrochemical proton gradient is equal to the rate of ATP consumption inside the cell. If the rate of ATP consumption is negligible compared to the velocity with which the ATPase equilibrates the proton motive force and intracellular ATP, ADP, and P_i, then

$$\frac{\Delta\bar{\mu}_{H^+}}{F} = \frac{\Delta G^{\circ\prime}}{nF} + \frac{2.3RT}{nF}\log\frac{[\mathrm{ATP}]}{[\mathrm{ADP}][\mathrm{P_i}]} \qquad (2)$$

In this equation $\Delta G^{\circ\prime}$ is the standard free energy of ATP hydrolysis and n is the number of protons transported per molecule of ATP synthesized or hydrolyzed. The sum of $\Delta G^{\circ\prime}$ and $2.3RT$ log $[[\mathrm{ATP}]/([\mathrm{ADP}][\mathrm{P_i}])]$ is often called the "phosphate potential" [e.g., Casadio et al. (1974), Leiser & Gromet-Elhanan (1977), and Azzone et al. (1978)] or the "phosphorylation potential" [e.g., Kell et al. (1978)]. It can be expressed in millivolts if it is divided by the Faraday constant F.

Recently, the phosphate potential and the electrochemical potential difference acting on protons have been compared in chromatophores (Casadio et al., 1974; Baccharini-Melandri et al., 1977; Leiser et al., 1977; Kell et al., 1978) and mitochondria (Nicholls, 1974; Azzone et al., 1978). Conflicting results were obtained. If the carotenoid band shift or the fluorescence increase of 8-anilinonaphthalenesulfonic acid, calibrated by K^+ diffusion potentials, was used for the measurement of the membrane potential, values more than twofold greater were found compared to the values obtained by ion distribution techniques [see Michels & Konings (1978) and Symons et al. (1979)]. Some authors therefore correlate phosphate potential and proton motive force with $n = 2$ under most conditions (Casadio et al., 1974; Baccharini-Melandri et al., 1977), whereas other workers need $n = 4$–5 (Kell et al., 1978). Nevertheless, one result was obtained which cannot be interpreted by the chemiosmotic hypothesis in a simple manner. Under steady-state conditions, uncouplers depress the proton motive force more than the phosphate potential, in both chromatophores (Baccharini-Melandri et al., 1977)

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[1] Abbreviations and symbols used: ΔpH, extracellular pH (pH_o) minus intracellular pH (pH_i); $\Delta\psi$, difference of the electrical potential across the cell membrane; basal salt, 4.3 M NaCl, 81 mM $MgSO_4$, and 27 mM KCl; $\Delta\bar{\mu}_{H^+}/F$, difference of the electrochemical potential of the proton or the proton motive force; n, number of protons translocated per molecule of ATP hydrolyzed or synthesized; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N*,*N*′-dicyclohexylcarbodiimide; DMO, 5,5-dimethyloxazolidine-2,4-dione; $TPMP^+$, triphenylmethylphosphonium ion.

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and mitochondria (Azzone et al., 1978).

In the preceding paper (Michel & Oesterhelt, 1980) we reported the measurement of the proton motive force and ATP, ADP, and phosphate levels in *Halobacterium halobium*. The results under anaerobic dark conditions could easily be explained by the presence of a reversible proton-translocating ATPase which equilibrates proton motive force, ATP, ADP, and phosphate very rapidly. In this communication we report experiments which were performed in order to correlate the light-induced increase of the proton motive force and phosphate potential.

Experimental Procedure

The materials and methods used have been described in detail previously (Michel & Oesterhelt, 1976, 1980; Hartmann & Oesterhelt, 1977).

For the measurement of membrane potential, pH gradient, and intracellular ATP and ADP concentrations as a function of irradiance, the cells were pretreated as follows. Cell suspensions were first pipetted onto silicone separating layers in centrifuge tubes, which were then stored in the dark anaerobically for 1 h. They were then illuminated for 10 min with the maximal irradiance (22 mW/cm^2) while in the centrifuge, incubated for another 5 min in the dark, and illuminated for 10 min with the desired irradiance. The accumulation of [^{14}C]TPMP$^+$ and [^{14}C]DMO in the cells was measured by determining the radioactivity in the cells after removal of exogenous material by centrifugation of the cells through the silicone separating layer. Since with this method bacterial pellets are only formed after passing the silicone separating layer and the probes cannot penetrate through the silicone separating layers, efflux of the probe ions in the pellets will not influence the results. Alternatively, 0.1-mL aliquots were taken for the determination of ATP, ADP, and inorganic phosphate. All determinations were carried out at room temperature (23 ± 2 °C) in duplicate.

Results and Discussion

Increase of Electrochemical Proton Gradient and ATP Level in Basal Salt at pH 6 and 8. According to the chemiosmotic hypothesis, generation of proton motive force is a prerequisite for ATP synthesis by a proton-translocating ATPase. Under conditions where illumination or oxidation of substrates leads to ATP synthesis, an enhancement of the proton motive force should be found, which in equilibrium (of the ATPase-catalyzed reaction) is as large as the enhancement of the phosphate potential, divided by nF. Under nonequilibrium conditions the increase of the proton motive force should be larger than that of the phosphate potential (divided by nF).

For verification of any correlation between the increase of phosphate potential and the increase of the proton motive force, cell suspensions were illuminated with different irradiances for 10 min and the membrane potential, pH gradient, and ATP, ADP, and P_i levels were determined during illumination. Surprisingly, at an extracellular pH of 6, illumination with irradiances of only 2 mW/cm^2 led to the maximal ATP concentration without any measurable enhancement of the membrane potential or pH gradient (Figure 1). Only irradiances that are larger than necessary for reaching the maximal ATP concentration cause an increase of membrane potential and pH gradient. For instance, in the experiment shown in Figure 1 the intracellular ATP and ADP concentrations at zero irradiance were 1.2 mmol/kg intracellular water and the phosphate concentration was about 60 mmol/kg of H_2O. The term 59 mV × log [[ATP]/([ADP][P_i])] is thus 72 mV in

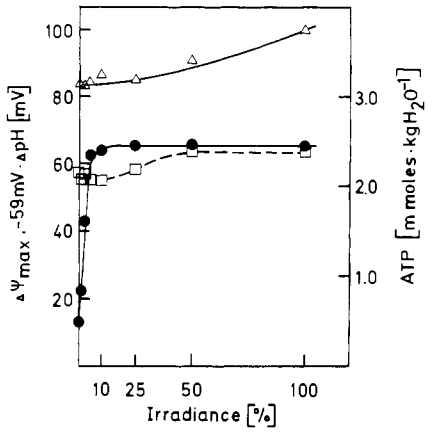


FIGURE 1: Dependence of $\Delta\psi_{max}$ (Δ), −59 mV × ΔpH (□), and the intracellular ATP concentration (●) on irradiance at pH 6.3. The cell suspensions (2.75 mg of protein per mL) in buffered basal salt (75 mM Tris–maleate, pH 6.3) contained 3.5 μM [^{12}C]TPMP$^+$ and 22 μM [^{14}C]DMO for the determination of the pH gradient, 22 μM [^{12}C]DMO and 3.5 μM [^{14}C]TPMP$^+$ for the determination of the membrane potential, and 22 μM [^{12}C]DMO and 3.5 μM [^{12}C]TPMP$^+$ for the determination of ATP, ADP, and P_i levels. 100% irradiance = 22 mW/cm^2; time of illumination was 10 min; pretreatment of the cell suspensions was as described under Experimental Procedure. For details of $\Delta\psi_{max}$, see footnote *c* of Table I.

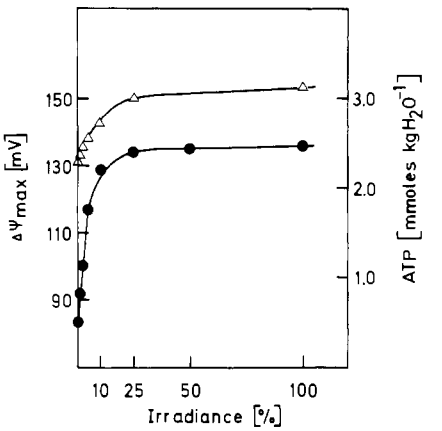


FIGURE 2: Dependence of $\Delta\psi_{max}$ (Δ) and intracellular ATP concentration (●) on the irradiance at pH 8.0. The experiment was performed as in Figure 1, except that the pH of the buffered basal salt was 8.0. The same cell suspension was used. For definition of $\Delta\psi_{max}$, see footnote *c* of Table I.

the absence of illumination. Within 10 min of illumination at an irradiance of 1.1 mW/cm^2, the ATP level was increased to 3.14 mmol/kg of H_2O, ADP decreased to less than 0.3 mmol/kg of H_2O, and phosphate remained essentially unchanged. The sum of ADP and ATP concentrations showed an increase at the expense of AMP (Oesterhelt et al., 1977). Thus, the term 59 mV × log [[ATP]/([ADP][P_i])] becomes 132 mV. Since with the low irradiance used no change of the intracellular pH was found, no change of the standard free energy of ATP hydrolysis occurred. Therefore, the total change of the phosphate potential was 60 mV. According to eq 1, an increase of 20 mV in $\Delta\psi$ + 59 mV × ΔpH should correspond to the increase of the phosphate potential if n = 3 or 15 mV if n = 4. Such an increase should have been detected but was not found. When the same experiment was performed at an extracellular pH of 8, higher irradiances were necessary to reach the maximal intracellular ATP concentration (Figure 2). Parallel to the increase of the ATP level, an increase of the membrane potential was found. With lower irradiances (up to 5 mW/cm^2), which do not influence the pH difference and thus the standard free energy of ATP hydrolysis,

Table I: Accumulation of $TPMP^+$ in Basal Salt ([NaCl] = 4.3 M) and in Basal Salt with Reduced NaCl Concentration (2.58 M) and the Influence of 10^{-6} M CCCP[a]

pH	illumination	$TPMP^+$ accumulation[b]	$\Delta\psi_{max}$[c] (mV)	$TPMP^+$ accumulation[b] with 10^{-6} M CCCP	$\Delta\psi_{min}$[c] (mV)
		(A) Basal Salt			
6.2	+	62	106	ND[d]	
	−	36	92	15	76
7.2	+	112	121	ND	
	−	72	110	23	102
8.2	+	342	150	ND	
	−	183	134	33	126
		(B) Reduced NaCl Concentration			
6.3	+	5	41	ND	
	−	5	41	5	0
7.2	+	7	50	ND	
	−	7	51	7	0
8.2	+	102	119	ND	
	−	75	111	23	100

[a] A concentrated cell suspension (13.8 mg of protein per mL) was diluted by dropwise addition of a threefold volume of (A) basal salt or (B) 1.43 M NaCl, 81 mM $MgSO_4$, and 27 mM KCl. 0.8-mL cell suspensions were buffered by addition of 0.2 mL of 375 mM Tris-maleate buffer in basal salt, pH 6.2, 7.2, or 8.2. Then 0.2-mL aliquots were incubated with 3.5 μM [^{14}C]$TPMP^+$ in centrifuge tubes for 1 h in the dark. For the cell suspensions with reduced NaCl concentrations pure silicone oil CR 500 (Wacker, Munich) was used as the separating layer. Half of the samples were illuminated for 10 min (irradiance 7.3 mW/cm^2) and centrifuged during illumination, and the other half were centrifuged in the dark. [b] $TPMP^+$ accumulation is given as $TPMP^+$ per kilogram of intracellular H_2O divided by $TPMP^+$ per kilogram of extracellular H_2O without correction for binding of $TPMP^+$ to membranes. [c] $\Delta\psi_{max}$ is calculated from the $TPMP^+$ accumulation without correction for uncoupler-sensitive binding to membranes, $\Delta\psi_{min}$ with correction [see Michel & Oesterhelt (1980)]. [d] ND = not determined.

the increase of the phosphate potential was compared to the increase of the membrane potential. In four different experiments the increase of the membrane potential and of 59 mV × log [[ATP]/([ADP][P_i])] was linearly related, and values for n could be estimated. The large variation obtained in the value of n (4.9 ± 1.6) is not surprising, since the increases of membrane potential and phosphate potential are obtained as differences of large numbers and subjected to large experimental errors. These estimations of n should therefore not be taken as quantitative measurements. Qualitatively, these results at pH 8 are in agreement with the presence of a rapidly equilibrating ATPase, and the parallel increase of phosphate potential and membrane potential would have been predicted by the chemiosmotic theory.

Changes of the Electrochemical Proton Gradient and ATP Level at Reduced NaCl Concentration. The lack of an increased difference in electrochemical proton potential when halobacterial cells are illuminated at low irradiance at pH 6.3 becomes even more prominent when the NaCl concentration of the basal salt (4.3 M NaCl, 81 mM $MgSO_4$, and 27 mM KCl) is reduced and not replaced by KCl or an osmotically active component. At pH 6.5 the dependence of the pH gradient and the ATP level on the irradiance is similar to that in basal salt, but there is no uncoupler-sensitive uptake of [^{14}C]$TPMP^+$ at the lower NaCl concentrations; a small (six- to sevenfold) accumulation in the experiment of Figure 3 could be accounted for by binding of the ion to the cell membrane. Nevertheless, the ATP synthesis was totally abolished by 10^{-5} M CCCP (data not shown).

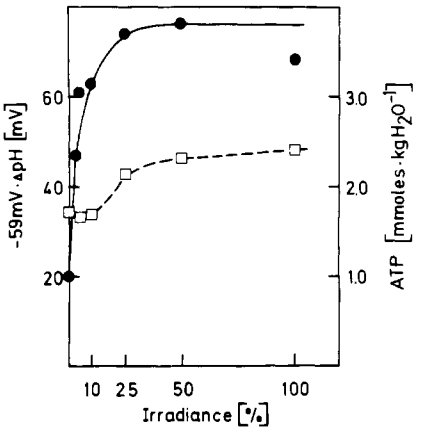


FIGURE 3: Dependence of −59 mV × ΔpH (□) and intracellular ATP concentration (●) on irradiance at pH 6.5 and reduced NaCl concentration (2.58 M). The experiment was done as in Figure 1, except that the NaCl concentration was only 2.58 M and the extracellular pH was 6.5.

Table II: $TPMP^+$ Accumulation at Reduced NaCl Concentration[a]

no.	[NaCl] (M)	additions	irradiance (mW/cm^2)	$TPMP^+$ accumulation[b]	$\Delta\psi_{max}$ (mV)[c]
A1	4.3		0	32	89
	4.3		7.3	39	94
A2	2.58	KCl	0	29	86
	2.58	KCl	7.3	52	101
B3	4.3		0	31	88
	4.3		22	98	117
B4	2.58		0	9[d]	
	2.58		22	8[d]	
B5	2.58	glycerol	0	8[d]	
	2.58	glycerol	22	42	96

[a] Cells were centrifuged down and resuspended in (A1) basal salt, (A2) 2.15 M NaCl, 2.18 M KCl, and 81 mM $MgSO_4$, and (B3-B5) basal salt (all at a protein concentration of 13.8 mg/mL). Aliquots were diluted (B3) with 3 volumes of basal salt, (B4) dropwise with 3 volumes of 1.433 M NaCl, 81 mM $MgSO_4$, and 27 mM KCl, and (B5) dropwise with 3 volumes of 1.433 M NaCl, 81 mM $MgSO_4$, 27 mM KCl, and 5.73 M glycerol. 0.2 mL of 375 mM Tris-maleate buffer in basal salt, pH 6.3, was added to 0.8-mL aliquots from A1, A2, and B3-B5. The resulting NaCl concentration is given in column 2. 0.2-mL aliquots of the cell suspensions were incubated for 1 h in the dark, then irradiated for 10 min, and spun down during illumination or were alternatively spun down prior to illumination. [b] Expressed as $TPMP^+$ per kilogram of intracellular H_2O divided by $TPMP^+$ per kilogram of extracellular H_2O without correction for binding of $TPMP^+$ to membranes. [c] For definition of $\Delta\psi_{max}$, see footnote *c* of Table I. [d] This $TPMP^+$ accumulation is not uncoupler sensitive and does not represent a membrane potential.

Table I compares the accumulation of $TPMP^+$ and the maximal membrane potential in basal salt and a basal salt with reduced (2.58 M) NaCl concentration. The cells at pH 8 still maintain an uncoupler-sensitive $TPMP^+$ accumulation corresponding to 110 mV, which is stimulated by light. To obtain hints on the possible reasons for the lack of $TPMP^+$ accumulation at pH 6–7 and reduced NaCl concentration, we omitted NaCl and replaced it by an equimolar amount of KCl (Table II). There then existed an uncoupler-sensitive $TPMP^+$ accumulation in the dark which was enhanced upon illumination. If the omitted NaCl was replaced by an isoosmolar amount of glycerol, then in the dark no uncoupler-sensitive $TPMP^+$ accumulation was found, but uptake of $TPMP^+$ was induced by light. (Unfortunately, no metabolically inert substance could be used instead of glycerol in the experiment; sucrose is not soluble in the high concentrations required,

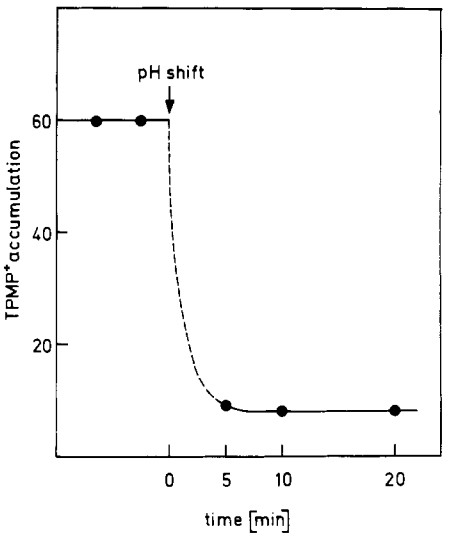


FIGURE 4: Reduction of $TPMP^+$ accumulation upon shifting the extracellular pH from 7.95 to 6.45 at reduced NaCl concentration (2.58 M). To 160-μL aliquots, containing 20 mM Tris–maleate buffer, pH 7.95, was added 40 μL of 375 mM Tris–maleate buffer, pH 6.3, in basal salt with reduced NaCl concentration. The resulting pH was 6.45. Centrifugation was performed at the indicated times. $TPMP^+$ accumulation is given as $TPMP^+$ per kilogram of intracellular water divided by $TPMP^+$ per kilogram of extracellular water without correction for binding of $TPMP^+$ to membranes.

whereas sorbitol causes cell lysis).

Therefore, the absence of $TPMP^+$ accumulation in the dark at reduced NaCl concentration seems to be an ionic strength effect and not a specific effect of the decrease of the Na^+ concentration. The latter could have been suggested from the existence of an H^+/Na^+ antiport with a stoichiometry greater than 1 which was proposed by Lanyi & MacDonald (1976).

Cells treated with DCCD showed a strong light-induced $TPMP^+$ accumulation, even after reduction of the NaCl concentration, comparable to that seen at high ionic strength (Michel & Oesterhelt, 1980). We could not measure this accumulation exactly, due to the fragility of DCCD-treated cells at reduced NaCl concentration. Since the centrifugation technique using impermeable silicone separating layers makes artifacts in the determination of $TPMP^+$ uptake unlikely, we looked for theoretical reasons to explain the absence of $TPMP^+$ uptake at pH 6 and low salt concentration. It would be possible for $TPMP^+$ not to reflect $\Delta\psi$ accurately if its permeability were reduced under certain conditions, which means that it would take a very long time for the ion to reach equilibrium. This was excluded by the pH shift experiment shown in Figure 4. When the external pH was suddenly decreased by 1.5 units, the $TPMP^+$ that had been accumulated in the dark was quickly extruded. Influx of H^+ following the pH shift is expected to decrease the outside-positive membrane potential, and $TPMP^+$ responds quickly to this change. Note that this experiment was done at reduced NaCl concentration and at pH 6–7.

Under some conditions the passive permeability of the Cl^- ion might become so high that the Cl^- ions determine the membrane potential. In this context we should mention that the NaCl concentration was reduced by dropwise dilution of a concentrated cell suspension in normal basal salt with a threefold volume of 1.433 M NaCl, 81 mM $MgSO_4$, and 27 mM KCl within 10 min. This procedure does not influence the rodlike shape of the cells, and the intracellular volume is enhanced by less than 10%. This means that upon dilution a massive outflow of Na^+, K^+, and Cl^- ions must have occurred, since otherwise an osmotic pressure of up to 50 atm would be created inside the cells. Therefore, upon dilution a high permeability for these ions must temporarily exist.

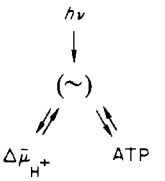
But even if the absence of a membrane potential at pH 6–7 and reduced NaCl concentration under anaerobic dark conditions is due to enhanced permeabilities of other ions, the increase of the ATP level upon illumination without a corresponding increase of membrane potential or pH difference is remarkable. This result cannot be easily interpreted by the chemiosmotic hypothesis.

A speculative explanation would be that the ATPase reaction is still in or close to equilibrium but that the ATPase reaction is not formulated in a complete and correct manner. There might be other components or states which take part in the ATPase reaction. Such other components or states could assimilate part of the energy from the electrochemical proton gradient. For instance, in a reaction scheme for the ATPase reaction

$$ATP + H_2O + H^+_i \rightleftharpoons ADP + P_i + H^+_o + X$$

in which X represents a conformational strain of the membrane or the ATPase molecule or different ionization (protonation) states of membrane components, X could store energy from the ATPase reaction.

Light-Induced ATP Synthesis in the Presence of Uncouplers. The increase of membrane potential and pH difference with irradiances far in excess of those required for maximal intracellular ATP production may indicate that ATP synthesis and formation of the proton motive force are parallel reactions, rather than that the proton motive force is the "precursor" of ATP synthesis. The difference between these two possibilities should be observed when the proton motive force is specifically affected, for instance, by proton conductors. If the proton motive force is the direct precursor of ATP production, then reduction of the proton motive force should abolish ATP synthesis. If the generation of the proton motive force and ATP synthesis lie on parallel routes as in the scheme



(3)

then the proton conductors should depress the proton motive force relatively more than the ATP level, under steady-state illumination. Such an experiment is shown in Table III.

At pH 8 a parallel increase of the proton motive force and the intracellular ATP level has been found, as shown in Figure 2. Table III shows the effects of the uncoupler CCCP (4 μM) on the ATP level and the accumulation of $TPMP^+$ in the dark and under steady-state illumination at this pH. The uncoupler depresses the accumulation of the indicator ion $TPMP^+$ to a low value which is presumably due to binding to $TPMP^+$ to halobacterial membranes (Michel, 1977).

Illumination of the cells in the presence of the uncoupler raises the ATP level to two-thirds of its maximal value, without any measurable increase of the membrane potential or the pH difference. This result is not in agreement with a strict interpretation of the chemiosmotic hypothesis but is compatible with a model (scheme 2) in which formation of the electrochemical transmembrane difference of the proton (proton motive force) and ATP synthesis are parallel reactions, both dissipating a different "energized state", whose nature is unknown. Such a model would also adequately describe the results of the preceding paper (Michel & Oesterhelt, 1980).

Table III: Light-Induced ATP Synthesis in the Presence of 4×10^{-6} M CCCP without a Measurable Electrochemical Proton Gradient[a]

CCCP	illumination	$TPMP^+$ accumulation[b]	$\Delta\psi_{max}$[c] (mV)	$-\Delta pH$	[ATP] (nmol/kg of H_2O)
−	−	112	121	0.2[d]	0.25
−	+	379	152	0.3[d]	3.15
+	−	43[e]	96	0.0[d]	0.22
+	+	42[e]	96	0.0[d]	2.03

[a] Cell suspensions (2.75 mg of protein per mL) in basal salt containing [^{12}C]DMO and [^{14}C]$TPMP^+$, or [^{14}C]DMO and [^{12}C]$TPMP^+$, or [^{12}C]DMO and [^{12}C]$TPMP^+$ have been used. The cell suspensions were strongly buffered with 75 mM Tris–maleate, pH 8.2. The irradiance was 22 mW/cm^2. Prior to or after 10 min of illumination, [^{14}C]DMO or [^{14}C]$TPMP^+$ uptake was determined or aliquots for ATP determination were taken. [b] Expressed as $TPMP^+$ per kilogram of intracellular H_2O divided by $TPMP^+$ per kilogram of extracellular H_2O without correction. [c] For definition of $\Delta\psi_{max}$, see footnote *c* of Table I. [d] Small pH gradients cannot be measured accurately. [e] This TPMP accumulation is due to binding of [^{14}C]$TPMP^+$ to cell membranes.

Similar results concerning the action of uncouplers on phosphate potential and proton motive force have been found with bacterial chromatophores (Baccharini-Melandri et al., 1977) and mitochondria (Azzone et al., 1978). Under steady-state conditions (continuous illumination or substrate plus oxygen plus ADP and P_i) the uncouplers depress the proton motive force more than the phosphate potential. In the paper of Baccharini-Melandri et al. (1977) arguments from the thermodynamics of irreversible processes have been used to explain such behavior in the framework of the chemiosmotic hypothesis, varying the apparent stoichiometric factor *n*, the number of protons translocated per molecule of ATP synthesized or hydrolyzed. The interpretation in the paper of Azzone et al. (1978) is that the proton motive force drives ATP synthesis within microscopic environments.

We think that the function of bacteriorhodopsin as a proton pump is compatible with the presence of ATP synthesis in the absence of a measurable proton motive force, if one assumes that a proton pumped by bacteriorhodopsin is used for ATP synthesis by a proton-translocating ATPase before it is released to the medium and enhances the proton motive force as measured in the bulk phases on either side of the membrane.

Only when the ATP level is high within the cell, or when perhaps the conduction of protons along the membrane is inhibited, as at pH 8, are the protons released to the medium. Unfortunately, very little is known about the properties of the electric double layer near the cell membrane, especially its conductivity for protons along the membrane.

Our interpretation coincides with the "electrodic" view of electron transport phosphorylation, which was published during revision of this manuscript (Kell, 1979). In this electrodic view working electrodes are compared to energy-transducing membranes. It emphasizes the existence of barriers to protons near the surface of energy-transducing membranes, as there are barriers to field-induced current flow in electrode/solution interphases.

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